Biological studies on the rivulariaceae

Kirkby, Susan M.

BIOLOGICAL STUDIES ON THE RIVULARIACEAE

By

Susan M. Kirkby (B.Sc. Dunelm)

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This thesis, which is entirely the result of my own work, has not been accepted for any degree, and is not being submitted concurrently in canditature for any other degree.

S. M. Kirkby

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ABSTRACT

This thesis reports a study on the variation of two genera of blue-green algae, Calothrix and Rivularia. The aspects chosen for detailed study were tapering and the hair. Before any comparative work could be undertaken it was necessary to develop ways of describing, in a quantitative manner, changes in the tapering and hair length. Tapering proved to be complex to describe in a satisfactory manner but four simple indices have been suggested. Measurements of individual trichomes have shown problems inherent in detailed studies of tapering, and the indices suggested by the author have been discussed in relation to this. Experimental studies on tapering have been concerned with elaborating the observations of previous authors that, in the presence of combined nitrogen, Calothrix and Gloeotrichia did not develop their characteristic taper. The effect of combined nitrogen on morphology was studied using the tapering indices.

Attempts to culture material with hairs proved difficult; however a few factors which affect the development of the hair have been described. A computer program was developed in order to summarise relationships between the occurrence of hairs and other morphological features which have been described in the literature.

For practical reasons it was often necessary to assign a name to members of the genus Rivularia; however in doing this many problems were encountered. To overcome some of these difficulties an objective method of identification was devised. This was used in particular to identify material collected in the field. A brief ecological survey of Rivularia was undertaken in order to study possible correlations between certain aspects of water chemistry and the occurrence of members of the genus.

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1. INTRODUCTION

1.1 Introduction to the family Rivulariaceae

The biology of tapered filaments has received relatively little study, in spite of the fact that this character delimits one of the main families of the blue-green algae, the Rivulariaceae. Because of the importance of tapering in classical taxonomy and the scarcity of information concerning its physiological basis, the author decided to investigate the character of 'tapering' and to study several aspects of the biology of the Rivulariaceae which may be closely associated with this. A broad approach to the problem was planned, in order to establish what are the key biological questions which should be asked before developing more intensive studies. A relatively extensive introduction to many aspects of the biology of the family has therefore been provided.

The Rivulariaceae are characterised by the possession of a tapered trichome during at least some stage of their developmental history. The trichome may or may not form a colourless hair. Trichomes may be simple or show false branching just below an intercalary heterocyst. Branching may occur in the interval between heterocysts, in which case the branches resemble those of Scytonema (Tilden 1910). Heterocyst(s), if present, may be basal or intercalary.

The family was first described by Agardh (1824). The genera of the Rivulariaceae were described in many of the early taxonomic works, including those of Rabenhorst (1865), Thuret (1875)

and Borzi (1882). The family was validated by Bornet and Flahault (1886), who recognised ten genera. Eight of these possessed a basal heterocyst: Brachytrichia, Calothrix, Dichothrix, Gloeotrichia, Isactis, Polythrix, Rivularia and Sacconema while the remaining two, Amphithrix and Leptochaete, had no heterocysts.

Hammatoidea and Tapinothrix were added to the family by Lemmermann (1910) and Geitler (1925, 1932, 1942). Homoeothrix was first described by Bornet and Flahault (1886), as a section on Calothrix. It was designated a new genus by Kirchner (1898). Raphidiopsis was included in the family by Fritsch and Rich (1929). Desikachary (1959) did not include this genus; however more recently Hill (1972) has again included it within the Rivulariaceae. Descriptions of the genera included in the family by Geitler (1932), i.e. excluding Brachytrichia and Raphidiopsis are given in Table 1.1.

The most useful account of the Rivulariaceae, which puts them into perspective with other blue-green algae, is still that of Fritsch (1945). There have been relatively few reviews dealing specifically with these organisms, so some topics only indirectly relevant to this thesis are referred to here. It is hoped that the present review and bibliographies will prove useful to future workers in this field. Based on this background information, specific aspects of the biology of the family were selected for study (Sections 1.3, 1.5).

1.2 Morphological variation in the Rivulariaceae

Morphological variation has long been recognised as a common

Table 1.1

Descriptions of genera of the Rivulariaceae (based on Geitler, 1932)

Genus	Authority	Date	Description
Amphithrix	Kützing	1843	Colony crust-like. Trichomes unbranched, erect, originating from a disc-like layer of laterally connecting cells. Heterocysts lacking, spores unknown. Hormogonia single or several.
Calothrix	Agardh	1824	Filaments single or in small groups, sometimes united in a cushion-like colony, mostly erect, more or less parallel; single, unbranched or rarely false branched. Sheath firm at least at base. Heterocysts mostly basal, sometimes intercalary. In some species the spore(s) is at the base of the filament, single or in small groups. Usually several homogonia in series.
Dichothrix	Zanardini	1858	Trichomes heaped together with many subdichotomous false branches. The bases of several false-branches (2-6) are often together in a common sheath. Spores not known.
Gloeotrichia	Agardh	1842	Filaments radial or more or less parallel, joined together in spherical or hemispherical form. Common colony mucilage. Often false branched. Sheath at base of filament firm, becoming slimey on the outside. Heterocysts basal and also intercalary, frequently at the base of false branches. Trichomes often with distinct trichothallic region. Spores at base of trichome single or sometimes several next to basal heterocyst. Hormogonia single or several.

Table 1.1 (continued)

Genus	Authority	Date	Description
Hammatoidea	West and West	1897	Trichome ending in long hair which grows out from taper at both ends. Firm sheath. Heterocysts lacking, spores unknown. Hormogonia present. Filaments mostly bent over so that both ends run more or less parallel.
Homoeothrix	Thuret Kirchner	1898	Trichomes undivided or false-branched at the base, seldom further up. Filaments erect, cushion-like and crusty forming a lawn. Heterocysts and spores lacking.
Isactis	Thuret	1875	Trichomes sparsely false-branched, ending in a hair. Filaments erect, parallel, in a mucilage sheath. Colony forms a soft, flat thallus.
Leptochaete	Borzi	1882	Filaments never branched, mostly joined together in crust-like colonies, single and appear to divide in three directions (Chroococcales). Heterocysts lacking, spores unknown. Hormogonia single or several.
Polythrix	Zanardini	1872	Similar to Dichothrix but much more radiating, to form a tuft.
Rivularia	Roth Agardh	1824	Trichomes unbranched or with more or less irregularly spaced false-branches. Filaments more or less radial or parallel, colony hemispherical or spherical, subsequently spread out. Old colonies often confluent and in common mucilage. Outer part of sheath more or less mucilagenous. Trichomes end in a hair and there is often a definite zone of trichothallic growth. Heterocysts basal or intercalary often at the base of a false-branch. Hormogonia single or in series from intercalary meristem. Spores absent.

Table 1.1 (continued)

Genus	Authority	Date	Description
Sacconema	Borzi	1882	Filaments small and 'bush-like' with much mucilage. Trichomes with few single cells or irregular false-branches. Thin cells in a thick sheath, tapering into a hair. Sheath very thick and sac-like, tapered or funnel-shaped, or in several pieces. Heterocysts basal, hormogonia, spores at base of filament.
Tapinothrix	Sauvageau	1892	Trichomes unbranched, tapered at the end, not ending in a hair consisting of cells from the trichome, but in a 'slime hair'. Heterocysts lacking, spores unknown, hormogonia present.

feature in the blue-green algae (Pearson and Kingsbury 1966). This has caused considerable taxonomic confusion, the major taxonomic works being based almost entirely on morphological differences, as illustrated by Forest and Khan (1972). In practice, difficulties encountered when naming members of the Rivulariaceae at the specific and occasionally the generic levels, have been described by Darley (1968). Koster (1961) attributed such difficulties to the lack of knowledge concerning the exact influence of external conditions on the growth and morphology of cells and colonies.

Such variation can arise in response to variation in the environmental conditions, both in the field and the laboratory (Pringsheim 1967, Koster 1961). It can also occur when several different cell types are derived from one genome (Carr and Bradley 1973). This later source of variation has particular relevance to the taxonomy of the Rivulariaceae. Single filaments of members of this family may contain up to four different cell types (see below). If the external conditions are such that one or several of these cell types cannot develop then considerable taxonomic confusion is produced.

1.21 Differentiation of the filament

The types of morphological variation described by Carr and Bradley (1973) are particularly noticeable in the Rivulariaceae, where up to four cell types may be present in a single filament. These are the normal vegetative cells, spores (akinetes), heterocysts

(all described below) and hair cells described in Section 1.32.

(i) Vegetative cells.

Relatively little information has been published concerning the cells of the Rivulariaceae. Several early authors, including Swellengrebel (1910) and Guilliermond (1933), studied the central structure of members of the group. Swellengrebel remarked on the lack of clear differentiation between the cytoplasm and nucleoplasm of Calothrix fusca.* Weber (1933) described in detail, changes in the vegetative cells and the heterocyst of C. fusca and C. braunii. Von Zastrow (1953) described the structure of cells of Gloeotrichia pisum and Calothrix marchica, as shown by several staining procedures. His work was mainly on the 'central substance', vacuolation and plasmolysis. The earlier work of Palla (1893) also described the central substance and vacuolisation of Gloeotrichia pisum. Several of these papers mentioned vacuolisation associated with the hair cells (Von Zastrow 1953, Weber 1933).

(ii) Spores.

From the taxonomic descriptions, two genera of the Rivulariaceae were defined as having spores, Calothrix and Gloeotrichia. All members of the genus Gloeotrichia, but only some species of Calothrix, possess spores at a certain stage of their development. The first record of a spore in a member of the genus Calothrix, C. crustacea, was provided by Borzi (1882). The second report, by Gomont (1895), described spore formation in a new species,

* A list of the authorities for each specific name is given in Appendix I.

C. stagnalis. Gomont (1895) compared the spore development in these two species and found that C. stagnalis did not form a long series of spores, as did C. crustacea, but that in both species, encystment proceeded from the base to the apex of the trichome. Gomont also reported that spores were produced when conditions were most favourable for C. stagnalis, while in C. crustacea they were formed under unfavourable conditions. Furthermore, he observed that there were two types of filaments, the first type were short and had hairs, while the second type were long and the end portion was lost in the production of hormogonia. Only the first type of filament possessed spores and Gomont suggested that if most of the cells' activity was used up in spore formation, then the system of vegetative development of the trichome might be hampered.

The mode of formation and germination of Gloeotrichia spores was studied fairly intensively by a number of workers including de Bary (1863), Bornet and Thuret (1880), Schwendener(1894), Geitler (1936), Palik (1941) and Desikachary (1946). A summary of these works has been given by Desikachary (1959).

(iii) Heterocysts.

Serbanescu (1966) described several aspects of the development of Gloeotrichia natans, including the two different stages in the life-cycle, which had been called the 'Pseudorivularia' stage and the 'Eugloeotrichia' stage by Poljansky (1930). In the first stage, the heterocyst at the base of the filament was reported to germinate and in the other there was a secondary meristematic zone, part of which formed new filaments. Apparently, germination of the

heterocyst, and subsequent development of the germling into a fully grown trichome, have been described only for seven species of the blue-green algae. The two earliest reports involved the non-Rivulariaceae, Nostoc commune (Geitler 1921b) and Tolypothrix elenkinii (Hollerbach 1923). The remaining reports concern members of the Rivulariaceae, Calothrix weberi (Steinecke 1932), Brachytrichia balani (Iyengar and Desikachary 1944), Gloeotrichia raciborskii and Rivularia manginii (Desikachary 1946) and the non-sporulating mutant clone of Gloeotrichia ghosei (Singh and Tiwari 1970).

Calothrix weberi was one of the few blue-green algae in which the majority of the stages in the development of a new trichome were followed (Steinecke 1932). The contents of the heterocyst divided by transverse division to produce a two-celled germling. The cell close to the pore of the heterocyst then squeezed out through the pore and by further divisions gradually grew into a mature filament. The second cell which remained inside, subsequently degenerated. These stages in germination differed from all of those reported so far, in that there were no other records of the upper cell emerging through the heterocyst pore, to form a new filament, and of the lower cell degenerating. Germination in Gloeotrichia raciborskii and Rivularia manginii (Desikachary 1946) agreed closely with observations on Nostoc commune (Geitler 1921b), in that the germling reached a four-celled stage in the heterocyst and then became free of the heterocyst. In these two members of the Rivulariaceae this process occurred by a widening of the heterocyst pore. Rejuvenation of the contents of the heterocyst were also described in Gloeotrichia raciborskii. Germination of G. ghosei (Singh and Tiwari 1970) was similar to that described for G. raciborskii.

Detailed accounts of the spatial arrangements of heterocysts were given by Fritsch (1951). The majority of heterocysts were of the terminal type. Those of Calothrix were similar to those of the Nostocaceae. In Dichothrix, the heterocysts tended to form successively at the base of the filaments, often losing their connections with the overlying cells at an early stage and sometimes failing to develop polar thickenings. The older heterocysts of Dichothrix were often widely separated from their filaments by a long connecting strand. The heterocysts of Gloeotrichia natans were spherical and were connected with the overlying vegetative cells by a course strand, which commonly had an encircling ring.

Based on observations of the close spatial relationship between the heterocyst and the spores, and the visible connection between them, Fritsch (1951) suggested that a spore forming substance was present or secreted from the heterocyst. Fogg (1949), suggested that in Anabaena cylindrica Lemm., the frequency of heterocysts was related to the concentration of combined nitrogen in the medium, the frequency of heterocysts being reduced in the presence of combined nitrogen (see also Section 1.31). Singh and Tiwari (1970) reported that in Gloeotrichia ghosei, ammoniacal nitrogen did not appear to influence the differentiation of successive crops of heterocysts and their subsequent germination.

1.22 Variation along a trichome

The presence of a variety of cell-types in a single trichome inevitably produces morphological variation along the length of an

individual trichome. Without exception, the diagrams of Rivularia and Gloeotrichia in the floras (Tilden 1910, Geitler 1932, Desikachary 1959) indicated a polarity within the colony. In every case there was a reduction in the diameter of the trichome towards the outside of the colony and heterocysts were at the basal end of the trichome, in the inner part of the colony.

There have been few studies of the variation between cells of a single filament. Haupt (1923) observed in Rivularia (= Gloeotrichia) pisum, that in a cross-section at the base of the filament there were large, peripheral cyanophycin granules, while in a cross-section a short distance from the base, there were no such granules.

Miller and Lang (1971) described changes in the structure of the cells along the trichomes of Gloeotrichia, as seen with the electron microscope. In the young cells the two membrane components of the thylakoids were contiguous, while in the older, more attenuated cells they became progressively more vacuolate. With increasing age, the number of polyglucoside granules decreased in number, while the number and size of the phycocyanin granules increased. The oldest cells lacked both types of inclusions and possessed single thylakoid membranes which appeared to bound remnants of intrathylakoidal cytoplasm.

Feldmann and Guglielmi (1973) described the vacuolisation of the thylakoids of the hair cells of Rivularia mesenterica. Here α-granules were present but cyanophycin granules were absent. With the decrease in the number of thylakoids, only nucleoplasm was left in the apical cells of the hair.

1.23 Morphological variation in response to environmental conditions

Apart from forms of morphological variation inherent in cell types, there have been several reports of variation in response to environmental conditions. Allen (1968) reported that the sheath of *Calothrix* became colourless, in response to a decrease in the light intensity. Anand (1937) referred to variation in the thickness of the sheath as a protection against desiccation and to reflect sunlight.

Darley (1968), in her laboratory studies, observed variations in the pattern of branching, characters of the sheath and development of the hair. *C. prolifera* was reported to form many more branches when it was cultured in Miquel's medium, than in its natural surroundings. *C. pulvinata*, cultured in the same medium, developed branching of the *Tolypothrix*-type, while *Calothrix crustacea* showed branching similar to that of *Scytonema*. Darley's culture work supported Anand's (1937) findings, and demonstrated that the thickness of the sheath was related to its importance in protecting the trichomes. *Calothrix pulvinata* and *C. scopulorum*, which had thick and often pigmented sheaths in nature, had thin colourless sheaths when they were grown in Miquel's medium. Differences in the hair existed between species which were exposed for long periods and those which were continually submerged. Trichomes of *C. pulvinata* cultured in aqueous medium possessed hairs at their apices, while *C. crustacea*, when adapted to an aqueous medium, also formed hairs. In 'aerial culture', hairs were not differentiated in these species (see also Section 1.32).

Several instances of morphological variation in Rivulariaceae have been related to symbiotic relationships. Geitler (1934) reported differences in the morphology of Dichothrix orsiniana when it was subcultured outside the lichen Placynthium nigrum. On soft agar (1.5%), hormogonia developed, while on firmer agar (3.0%), longer filaments with hairs developed (see also Section 1.32).

Walsh (1965), observed irregular development of Calothrix sp. in the lichen Lichenes imperfecti. He recorded many trichomes without terminal hairs and although he commented on the possibility of this variation being genetically determined, he suggested that it was more likely to be due to the symbiotic relationship. He also attributed variability in the occurrence, shape and size of heterocysts to the presence of fungal hyphae.

1.24 Taxonomic implications of morphological variation

As mentioned in Section 1.2 much taxonomic confusion has arisen due to the basic taxonomic works being based on morphological differences (Forest and Khan 1970). Problems in the identification of Rivulariaceae have been encountered frequently. Hulbary (1954) commented on the lack of suitable keys for Calothrix and there are many cases where identification is based on a range of cell widths, which overlap between species (Geitler 1932, Tilden 1910). Malmeström (1972) described difficulties in distinguishing C. pulvinata and C. scopulorum.

Further problems arose when some of the different cell-types were absent and there was no way of telling a priori whether the structure was truely absent, or whether conditions were such as to

make differentiation impossible. Several taxonomists have questioned the separation of the two genera, Gloeotrichia and Rivularia, the distinction being based on the presence of spores in Gloeotrichia and their absence in the genus Rivularia. Geitler (1942), Fritsch (1945), Elenkin (1949) and Desikachary (1959) were among those who separated the two genera, while Kirchner (1898), Forti (1907) Tilden (1910) and Lemmermann (1910) classified them both under the name Rivularia.

The type of confusion which arose was illustrated in an early report on the determination of some of the bloom-forming algae by Bornet and Flahault (1884). They described several instances where trichomes without spores were found and as a consequence it was difficult to assign a generic name (Section 1.44). Singh and Tiwari (1970) obtained a non-sporulating clone of Gloeotrichia ghosei and as a result of their work questioned the validity of the genus Rivularia. They suggested that species which were included under Rivularia were non-sporulating forms of Gloeotrichia.

A similar situation existed with regard to the genus Homoeothrix which is distinguished (along with several other genera), by its lack of heterocysts. Homoeothrix was originally included within the genus Calothrix by Thuret (1875), from the point of view of being a 'Calothrix without a heterocyst'. Puymaly (1957) suggested that the basal cells of Homoeothrix were associated with attachment to a substratum in a similar way to the heterocysts of other genera.

In addition to such difficulties there are the problems that material may not contain all of the stages of the life-cycle necessary

for identification and that basal heterocysts may be lost or damaged during collecting.

1.25 Physiological aspects of morphological variation

Desikachary (1970) and Pringsheim (1967), have both pointed out the great need for growing organisms in culture, in strictly defined media, so that the possible variation of a particular taxonomic character could be determined.

Pearson and Kingsbury (1966) observed considerable variability in Calothrix membranacea compared to several other blue-green algae. Interpretation of their results was difficult as many characteristics of the alga varied widely, even among filaments in the same culture vessel. They cultured C. membranacea in several growth media to compare the effect of different mineral formulations. Growth did not occur in Knop's solution. Growth occurred in modifications of Chu 10 medium (Chu 1942) and in variations of Chu's (1942) 10 medium designed by Gerloff, Fitzgerald and Skoog (1950), best growth occurring in the former. Growth was slow in a modified Detmer's solution (Bold 1942), a modified Bristol's solution employing trace elements of Chantanachat and Bold (1962) and in soil water medium (Pringsheim 1946). Filaments varied from long, entangled or 'ropey', to short and broken. Hardly any cultural correlations appeared to exist, except that the degree of fragmentation was greatest in older control cultures. Unusual, irregular shaped cells were also present in many of the cultures, although they did not appear to correlate with any cultural characteristics. Location of the heterocysts and attenuation

of the trichomes were other variable characteristics showing no relationship to one another or to any particular condition of culture.

Darley (1968), in her discussion of variation in members of the Rivulariaceae, reported on the work of Poljansky (1928), which indicated that when C. elenkinii was cultured on agar, there were several variations in morphology including the disappearance of the hair.

The effect of specific substances on the morphology of the Rivulariaceae has been studied by few workers. Fay, Stewart, Walsby and Fogg (1968) described a change in the morphology of species of Calothrix and Gloeotrichia, as follows: 'when cultured with ammonia, the length of the filaments were not restricted (in this way) and the trichomes were of uniform appearance throughout'. Mitra (1965) described the lack of heterocyst induction by a variety of factors, as long as the nitrogen level in the medium remained above a critical level.

1.26 Nitrogen fixation studies

The majority of the experimental studies on members of the Rivulariaceae have been related to their ability to fix atmospheric nitrogen. The first species of the Rivulariaceae shown to be capable of fixing atmospheric nitrogen was C. brevissima (Watanabe 1951a). Since then this ability has been demonstrated in several members of the family, a list of these being given in Table 1.2. C. scopulorum was the first blue-green alga isolated from a marine habitat to show nitrogen fixation (Stewart 1962). Stewart (1964)

Table 1.2

Nitrogen fixation in the Rivulariaceae

Organism	Reference
Calothrix aeruginea	Stewart (1971)
C. antarctica	Saubert and Grobbelaar (1962)
C. brevissima	Watanabe (1951a)
C. clavata	Saubert and Grobbelaar (1962)
C. confervoides	Stewart (1971)
C. crustacea	Stewart (1971)
C. elenkinii	Taha and El Refai (1963)
C. parietina	Allen (1952)
C. scopulorum	Stewart (1962)
Dichothrix fucicola	Carpenter (1972)
Gloeotrichia echinulata	Williams and Burris (1952)
Rivularia atra	Stewart (1971)
R. biasolettiana	Stewart (1971)

provided the first demonstration of nitrogen fixation in a marine habitat, again using C. scopulorum. His field studies in subsequent years (Stewart, 1965, 1967a), using N^{15}, confirmed that C. scopulorum could fix nitrogen in the field and in laboratory culture. His data suggested that the alga contributed considerable quantities of fixed nitrogen to the environment. Fixation over a year showed a well defined seasonal variation, with spring and autumn maxima and little fixation during summer and winter. Stewart (1967a) also demonstrated that nitrogen fixation in C. scopulorum was reduced by 86% when it was grown at an initial N level of 50 mg l^{-1} NH_4-N.

Jones and Stewart (1969a), showed that C. scopulorum could grow and fix nitrogen over a wide variety of environmental conditions, with optima near to 5‰ salinity, 25°C, 8000 lux and pH 8.5. Under all conditions studied, extracellular nitrogen was liberated into the growth medium. In general, the greater the quantity of nitrogen fixed by Calothrix, the greater the quantity of extracellular nitrogen liberated. Also the nearer the environmental conditions were to the optimum for growth, the shorter the time taken for the percentage of extracellular nitrogen to decrease and for exponential growth to begin.

These facts are significant from the point of view of the ecological importance of the group. In their natural habitat, the factors mentioned above are in a state of continual change and so the liberation of extracellular nitrogen may be relatively high. The ability of other organisms to use this as a source of nitrogen was demonstrated by Jones and Stewart (1969a).

Estimates of the amount of nitrogen fixed by members of the Rivulariaceae have been made on a number of occasions. Williams and Burris (1952) estimated that in 5 days, the % cell-N from N^{15} was 43-50% for C. parietina and that 18% of the cell-N of Gloeotrichia echinulata was fixed in 30 days.

Watanabe (1951a) studied the influence of several blue-green algae on the production of rice crops and found that although Tolypothrix tenuis was superior to Calothrix brevissima in its ability to fix atmospheric nitrogen, these two algae exerted an almost equal influence on the growth of rice. However, he found that the quantity of nitrogen containing compounds secreted into the medium was low in the case of Tolypothrix tenuis, and comparatively high in Calothrix brevissima. Watanabe examined the amino acids in the algal cells and in the external solution, and demonstrated that although both species contained a wide range of amino acids, C. brevissima was the only one to secrete amino acids into the medium.

1.3 Tapering and the hair

The ability to form a tapered trichome, which may or may not form a colourless hair, during at least some stage of the life-cycle, is a characteristic of the Rivulariaceae. Little is known at present, about tapering and hair development and the relationships between them are not understood (Section 1.1, 1.23, 1.25). As much of the work described in the text is concerned with these two characters, more detailed literature reviews relevant to these characters are given.

1.31 Tapering

The term "tapering" has been used in many of the early taxonomic works (Agardh 1824, Borzi 1882, de Toni 1907) but none of these works give a formal definition of the term. It is assumed, therefore that they used it in the general concept of a gradual reduction in the diameter of the trichome. Consultation of the diagrams in taxonomic works and specified descriptions, indicated that there were no exceptions to this as a universal character of the family Rivulariaceae. In some species however, tapering has been described as slight e.g. C. membranacea (Geitler 1932). In most genera which have heterocysts and where the trichomes tapered towards one end only, the heterocyst was at the widest end (see also p. 20). In one genus, Hammatoidea, the trichomes tapered towards both ends.

The Rivulariaceae produced hormogonia (Fritsch 1945) which were short, parallel, motile trichomes with an indistinct sheath. Obviously if this stage in development was observed, it would not show the characteristic tapering of the family.

As mentioned previously (Section 1.25), there has been little experimental work concerning factors which may control tapering, or cause changes in the expression of this character. In this context, it is relevant to repeat the findings of Fay, Stewart, Walsby and Fogg (1968), which were as follows:

"growth of Calothrix and Gloeotrichia occurring in the cells near the basal heterocyst. ... as the vegetative cells are displaced to the distal end of the filament, they narrow, vacuolate and finally

die off, forming a colourless hair. ... suggested that the cells depend on a substance emanating from the heterocyst and passing from cell to cell. ...the substance might be nitrogenous, because when cultured with ammonia, the length of the filaments was not restricted (in this way) and the trichomes were of uniform appearance throughout". Because of the lack of information concerning this relatively important and distinct character, used at the family level of classification, the effect of combined nitrogen on tapering was studied further (Section 1.5).

1.32 Hair formation

In many species of Rivulariaceae, the cells near the narrow end of the trichome have been reported to form a hair (Geitler 1932, Fritsch 1945, 1951). Bornet and Flahault (1886) described the hair as a "series of narrow cells, elongated,colourless, containing very little protoplasm and which were not capable of further growth." Fritsch (1945), commented on the evident vacuolation which was characteristic of developing hairs and also mentioned that such hairs were present frequently in Scytonema and Stigonema. Bharadwaja (1933), described the attenuation of trichomes in Scytonema simplex and two species of Tolypothrix, in a way similar to those given above for the Rivulariaceae. Bharadwaja also mentioned the gradual narrowing which often accompanied the elongation of cells, and that when conditions were very acute, they became elongated soon after they were formed.

Tilden (1910) and Fritsch (1945) described the hair cells as being colourless and having long, slender, straight walls and containing little protoplasm. Both authors thought that the evident vacuolation which occurred in older cells was an indication that the hair cells were moribund and incapable of further growth. Fritsch (1945) attributed the yellowing of these cells to a loss of pigments other than carotene. Darley (1968) also described the hair as consisting of long narrow cells in which the contents decreased progressively and were replaced by large vacuoles. There are however no quantitative measurements on the pigment changes along the trichomes of the Rivulariaceae.

Recently, several workers have suggested that the hair cells might not be moribund. Fuhs (1973) suggested that the hair cells of Gloeotrichia which extended to the periphery of the colony and beyond, were the cells likely to suffer ~~from~~ least from nutrient limitations. Ueda (1971) studied the DNA content of cells of Calothrix braunii and reported that although the cell volume decreased from base to apex, the DNA content remained similar. In the transition zone between normal cells and hair cells the DNA content was less. Ueda suggested that cell division was taking place, without the preceding doubling in the DNA content and that a degenerative process resulting in the formation of plasma vacuolation could be observed in the hair cells. Wolk (1973) reported that the hair cells of C. braunii contained $\frac{1}{5} - \frac{2}{5}$ as much DNA as the actively growing vegetative cells.

Darley (1968)studied the variation in the hairs of several marine species of Calothrix, Isactis and Rivularia. She described

differences between Rivulariaceae which were out of water for long periods and those which were continuously submerged. Those such as Calothrix pulvinata,which were exposed, had a rounded terminal cell, while those which were submerged had a long hair. When algae from both of these types of habitat were kept submerged, in the laboratory, they developed hairs. After liberation of hormogonia those which were normally found exposed, did not reform a hair, while those which were normally submerged did reform hairs. Darley concluded that aerial conditions were not suitable for the development of hairs.

Jones (1967) noted the absence of the characteristic hair of C. scopulorum, when the salinity of the medium in which it was cultured was greater than 35‰. He also commented that in its exponential phase of growth, C. scopulorum was in a constant hormogonial condition. Presumably this indicates that there was no attenuation of the trichome, and that the hair was absent. Poljansky (in Darley 1968) also reported variations and the disappearance of the hair when C. elenkinii was cultured on agar with Knops-Fe medium.

A further situation in which variation in the presence of a hair occurs, is associated with symbiosis (see also Section 1.23). Geitler (1934) noticed that when Dichothrix orsiniana formed the algal symbiont of the lichen Placynthium nigrum, the development of hairs was completely suppressed. Furthermore the polarity of the filaments was lost and the growth greatly reduced. When the alga was cultured the growth form which developed depended on the medium used (1.5% or 3% soil agar, alkaline Knop's agar or soil

solution). On soft agar hormogonia were formed, while on firmer agar and in soil solution long filaments with hairs developed.

1.33 Meristems and development

It is necessary to mention briefly several aspects of the growth and development of trichomes of the Rivulariaceae, which may aid in understanding the development of tapering and the hair. Schwendener (1894) and Geitler (1936) described how in many Rivulariaceae, growth was trichothallic, the meristem being situated at the base of the hair. However De Bary (1836), Palla (1893) and Weber (1933) described division occurring in the basal cells. Cells in the meristematically active region(s) of the trichome give rise to yet another form of morphological variation within filaments (Section 1.22). Fay, Stewart, Walsby and Fogg (1968) have also described growth in the basal region of filaments of Calothrix and Gloeotrichia.

Fritsch (1945) described how the hormogonia were normally produced from the part of the trichome beneath the terminal hair, which was shed as they developed. During the development of a tapered trichome from a hormogonium, the heterocyst and taper appeared at approximately the same time. In some cases however, Fritsch (1945) described the formation of tapering as secondary; two new heterocysts differentiated near the centre of a trichome, which then broke between these two heterocysts, resulting in two tapered trichomes. Although little is known about the formation of a tapered trichome from a parallel hormogonium, the above facts must be borne in mind when describing changes in trichome shape.

1.4 The ecology of Rivulariaceae

1.41 Introduction

Members of the Rivulariaceae are found primarily in mountainous districts of the British Isles, particularly in streams and on dripping rocks in limestone regions, but they occur sparingly in lower regions (West and Fritsch, 1927). They also form a definite narrow zone on rocks in the littoral regions of lakes (Fritsch 1931, 1945), and are important in the littoral zone of marine habitats (Fritsch, 1945). The three works quoted above provide a useful summary of early ecological literature of the group, and give references to several British localities.

For completeness and to bring together as much of the background information as possible, reviews of epilithic freshwater Rivulariaceae, marine species and 'bloom-forming' species are given below. Records of the occurrence of Rivulariaceae are available from many areas, these being listed as a bibliography in Appendix III.

1.42 Epilithic freshwater forms

Of the few reported sites of Rivulariaceae in Britain, most concern upland regions. Rivulariaceae from Malham were described briefly by Lund (1961). Godward (1937) recorded the presence of Homoeothrix in Windermere and West and Fritsch (1927) reported the following:

Amphithrix janthina from wet rocks in W. Yorkshire

Calothrix parietina from Arncliffe, W. Yorkshire

Dichothrix interrupta from Slieve, Donard, Ireland

Hammatoidea normanii from Dartmoor, Devon

Rivularia biasolettiana from Arncliffe, W. Yorkshire

R. minutula, Chippenham Fen, Cambridge

From these few reports there is some indication that the genus Rivularia is rare. The situation indicated for the continent is different in that there are many records of Rivularia associated with fast flowing upland streams (Jaag 1945, Kann 1966, Golubić 1967). In many instances the group was associated with the deposition of calcium carbonate. Golubić (1967) quoted several of the early works on the deposition of carbonate and gave detailed phytosociological accounts of the occurrence of the family. In a recent review, Golubić (1973) described many aspects of calcium carbonate deposition, including the role of certain members of the Rivulariaceae, in particular R. haematites. The algae of calcareous rocks of Britain have been described by Fritsch (1950).

Recently Hughes and Whitton (1972) studied several calcareous streams in the north of England and found that Rivularia was abundant in parts of this area. The present author received a number of reports which suggested that Rivularia was also abundant in other streams in this area and in certain other parts of Britain. This information provided the background data for a more detailed ecological survey of the distribution of Rivularia.

1.43 Marine forms

In spite of the relatively large amount of literature concerning marine Rivulariaceae, a relatively short review related to their ecology is given here. Calothrix is the dominant intertidal, epilithic blue-green alga on temperate shores (Fritsch, 1945). The importance of Rivulariaceae in marine habitats

was described by Anand (1937) in a detailed account of the communities of British chalk cliffs. C. pulvinata var. prostrata and Rivularia atra were present on the chrysophyte belt on exposed cliffs, which were sprayed by the sea, but which were never submerged. The Calothrix community was essentially endophytic, the blue-green algae inhabiting the chrysophyte mat and causing a black colouration. The Calothrix was found all the year round, but was more obvious in the summer. Calothrix was seldom found in tunnels and caves and seemed to prefer exposed situations. A further example of its preference for good illumination was furnished by the fact that the filaments were only found in the upper parts of the chrysophyte mat. The mucilagenous character of the mat was thought to protect the Calothrix from desiccation.

The Rivularia community was always found well above high water mark (sometimes extending into the chrysophyte zone) and the dominant Rivularia was apparently intolerant of frequent submersion. The community showed a maximum development in winter, when abundant hormogonia production took place, while in the summer the plants became shrivelled and less conspicuous. Colonies usually appeared on freshly exposed surfaces, with only a thin chrysophyte covering. Dichothrix gypsophila sometimes occurred in the chrysophyte mats between the Rivularia colonies.

Anand also recorded extensive growths of Calothrix littoralis just above the high water mark, associated with Pleurocapsa and Plectonema. This constituted a distinct community, so far described only by Setchell and Gardner (1903), from the Pacific. Calothrix pulvinata was recorded in the chrysophyte community, but on a partly shaded surface. Grizbergen (1925) described a similar community

but with C. scopulorum in the upper zone. Carter (1932, 1933a, 1933b) described the occurrence of Rivularia in a salt marsh in Essex. The colonies were conspicuous for most of the year but were more so during wet winter months. The colonies adhered very closely to the surface of the soil so that in general they gave protection against erosion. C. scopulorum, one of the organisms used for this work, was isolated by Stewart from a rocky shore in W. Kilbride (Stewart 1962).

1.44 Planktonic forms

A few of the Rivulariaceae are planktonic, particularly the members of the genus Gloeotrichia. Some of these such as G. echinulata are truely planktonic, while others are found in the plankton only during part of their life cycle. There is only one truly planktonic Rivularia, R. planctonica and no members of the other genera are normally found free floating.

Several early reports on the occurrence of the Rivulariaceae describe those which form water-blooms. Bornet and Flahaut (1884) gave the first record of a floating Rivularia, which was discovered in 1804 by Rev. Davis, in a lake on the Isle of Anglesey. Such early observations illustrated the difficulties in naming Rivulariaceae e.g. Dikie observed some plants in a region near Aberdeen, which he named Rivularia echinulata. However once spores began to develop Dikie suggested the name Gloeotrichia pisum for the material. Bornet and Flahault suggested that Rivulariaceae associated with water-blooms should be assigned to the genus Gloeotrichia.

More recent records of bloom-forming Rivulariaceae, mainly associated with the breaking of the Meres, have been described by Sinker (1962), Wilson (1966) and Reynolds (1971). The ecology of Gloeotrichia echinulata was described by Roelofs and Oglesby (1970). There have been frequent reports of Gloeotrichia from warmer environments (Appendix III). These include a report of G. natans from a thermal spring in Japan (Yoneda 1952), where it was found at 47°C, which is apparently the highest recorded temperature for a Gloeotrichia.

1.5 Aims of research

As stated in Section 1.1, tapering and frequent development of a colourless hair, in the Rivulariaceae, have not been studied in detail. Very little is known about these characters in response to environmental conditions and ageing, yet tapering is an important character in the taxonomy of the Rivulariaceae. The aims of the present research were therefore, to study these characters in more detail. Before any comparative work was possible, it was necessary to define tapering and hair, and to devise a quantitative method of describing tapering (Chapter 3).

This necessitated a broad approach to the problem in order that the author could become aware of the possible range of variation in these characters. This knowledge was obtained by reading the descriptions in the floras and observing and measuring material in the laboratory.

Experimental studies were undertaken with the aim of finding specific causes for tapering and hair development. The experimental

work was designed mainly to elaborate the comments of Fay, Stewart, Walsby and Fogg (1968), concerning the loss of tapering in the presence of combined nitrogen (Chapter 4).

Observations of laboratory cultures and field organisms illustrated the wide variation within the Rivulariaceae. While studying field material, many problems were encountered in keying-down the organisms, using conventional floras. The main reasons for this were the variation shown by the filaments in any one colony and the large element of subjective judgement involved in identification. It was considered useful therefore, to develop an alternative, objective technique for identifying organisms (Chapter 5). Developing a standard recording procedure is in itself likely to be useful if the present trend in the use of the numerical taxonomic approach is continued.

A considerable number of personal reports, concerning the distribution of Rivularia were given to the author. It was hoped that by visiting these areas and analysing the water, a more detailed account of the distribution of Rivularia, in relation to water chemistry, would emerge from these studies.

The study was restricted to two genera, Calothrix and Rivularia. As only species of Calothrix were available in axenic culture, most of the experimental work was confined to representatives of this genus. Furthermore it was relatively easy to culture members of this genus in the laboratory and they do not have the colony structure of the Rivularia . . . which adds many complications to the experimental work. However, in the ecological work, members of the genus Rivularia

were the main study organisms. The macroscopic growth form of this group meant that it was easy to find in the field; a process which would have been difficult if Calothrix had been studied in the field.

Although it was impractical to study more than two genera, this will inevitably cause difficulties when drawing general conclusions, and this fact should be borne in mind throughout this work.

2 MATERIALS AND METHODS

2.1 List of abbreviations in text.

^{o}C	=	degrees Celsius
EDTA	=	ethylenediaminetetra-acetic acid
g	=	gram
h	=	hour
l	=	litre
µg	=	microgram
µm	=	micrometre
mg	=	milligram
ml	=	millilitre
mm	=	millimetre
min	=	minute
M	=	molar
nm	=	nanometre
OD	=	optical density
u.v.	=	ultra-violet
w/v	=	weight per volume

2.2 Algal materials

Three types of material were used during the present study, axenic cultures, unialgal cultures and field material. The majority of the experimental work made use of axenic cultures (Sections 4.2 and 4.3). The Durham culture numbers are employed in the text to

identify each strain, which does not have a specific name.

Axenic and unialgal cultures

Algal cultures were obtained from several Culture Collections and from individual people. Details of the cultures and their origins are given in Table 2.1.

Tests were made on the purity of all axenic cultures prior to their use for experimental purposes. These tests were carried out using beef peptone agar, malt extract agar and yeast agar, as described by Khoja (1973). All material was examined microscopically. As a further check, a 1% sucrose solution was added to an old flask of the material and after several days the culture checked for bacterial growth.

Field material

Field material was collected by the author and brought back to the laboratory in polythene bottles or bags. Material to be used for experiments was stored at 4°C and used within 24 h. Observations described in Chapter 3 were made using samples of Rivularia from four sites:

(1) Gordale Beck (SD 912656)

(2) Slapestone Sike (NY 815304)

(3) Sunbiggin flush (NY 671077)

(4) Tulach Hill (NN 869644)

In addition samples of Gloeotrichia from Brasside (NZ 292454), Llangorse (SD 127270) and Sunbiggin (NY 670075) were also used.

Table 2.1 Details of cultures

Species	Source of culture	Culture number at source	Durham culture number	Isolator	Habitat
Calothrix brevissima	Cambridge	1410-7	D 156	Watanabe 1951	Paddy field mud, Palau Island
	Tokyo	M-7	D 276		
Calothrix desertica	Göttingen		D 270	Schwabe	Fine sand near La Portada (Antofagasta, Chile)
Calothrix elenkinii	From R.N. Singh, Banaras		D 268		
Calothrix fusca	From R.N.Singh, Banaras		D 269		
Calothrix gracilis	Tokyo	M-55	D 274	Ishikawa	
Calothrix scopulorum	Cambridge	1410-5	D 256	Stewart 1962	Upper and supra-littoral, on west coast of Scotland
Calothrix thermalis	Gif-sur-Yvette	M-13/1	D 266		From warm water at Dax (S.W.France)
Calothrix viguieri	Cambridge	1410-6*1	D 253	Komárek	Cuban mangrove swamp
Calothrix sp.	Gottingen	1410-2*2	D 255	Straut	
Calothrix sp.	Indiana	B1827	D 258	Pant	India
Calothrix sp.	Gif-sur-Yvette	M-13	D 267		Soil

*1 This is equivalent to the culture kept at Trebon (KOM. 64/44). Information from Trebon stated that it was found on tree rind near Rio Arimas

*2 Cross-checking suggested that this species was equivalent to Fremyella diplosiphon

Each field sample collected was given a code number for computational purposes (Appendix IV).

2.3 Apparatus

(i) Glassware

All glassware used was of Pyrex brand.

(ii) Apparatus used for growing cultures

Material was cultured in conical flasks, stoppered with non-absorbent cotton wool plugs (Best quality C249, Robinson and Sons, Ltd., Chesterfield). The flasks were kept on shelves in growth rooms or in shake tanks, both with constant light and temperature regimes. Light was supplied continuously through 24 h, by warm white fluorescent tubes. For the majority of the experimental work the flasks were kept in the shake tanks and were moved through a horizontal distance of 20 mm, 72 times $min.^{-1}$

(iii) Measurement of physical features

The light intensity was measured with an EEL Lightmaster photometer, at the surface of the water in the shake tanks, or at the plane of the middle of the medium in the growth flasks and orientated at right angles to the source of light. Light intensity was expressed in lux.

Temperature was controlled thermostatically and was expressed in degrees Celsius.

(iv) pH measurements

Measurements of pH were made in the laboratory using a PYE Dynacap pH meter (Model 291), and in the field using a PYE Unicam pH meter (Model 293).

2.4 Chemicals

All of the chemicals used for making inorganic media were of Analar grade with the exception of $Ca(NO_3)_2$. Chemicals were supplied by the British Drug House Ltd. Non-analar sodium-β-glycerophosphate was used as a source of organic phosphate.

2.5 Media

During the early experimental work, media were prepared using distilled water, however during the later stages, deionised distilled water was used. Fresh media were prepared every time the algae were subcultured. Stock solutions were added to approximately 900 ml of water, the chelating agent, ethylenediaminetetra-acetic acid (EDTA) and the micro-elements being added last. In order to minimise the precipitation of phosphate in AD medium, the phosphate was made up and autoclaved separately. This was then added to sterilised media which had cooled for several hours. Solid media were prepared by boiling the liquid media (with phosphate added) and then adding Ion Agar at a concentration of 1.5% (w/v).

Several growth media were used during this study, the chemical compositions of which are given in Table 2.2. For most of the experimental work, a medium lacking combined nitrogen, modified from Allen and Arnon (1955), was used. In the remainder of the text, media lacking combined nitrogen will be designated '- N media' and media containing combined nitrogen, ' + N media'. The experimental medium modified from Allen and Arnon (1955) will be designated 'AD', and may be compared with the original medium in Table 2.2.

Table 2.2 Media composition, concentrations in mg l^{-1}

salt used	medium of Allen & Arnon (1955)	'AD' medium	ASM + N medium of Gorham et al. (1964)	ASM - N medium of Gorham et al. (1964)	'modified medium Z' of Staub (1961)	V_{37} medium of Provasoli (1957)
K_2HPO_4	348.36	250.0	8.7	8.7	31.0	25.0
$MgSO_4.7H_2O$	246.48	200.0	49.0	49.0	25.0	-
$MgCl_2.6H_2O$	-	-	41.0	41.0	-	7550.0
$CaCl_2.2H_2O$	73.51	66.2	29.0	29.0	-	380.0
NaCl	233.76	230.0	-	117.0	320.5	45085.0
Na_2HPO_4	-	-	7.05	7.05	-	-
$NaNO_3$	-	-	170.0	-	-	-
$NaCO_3$	-	-	-	-	21.0	-
K_2SO_4	-	-	-	-	-	1300.0
Fe (as EDTA complex)	4.0	4.0	-	-	-	10.0
Na_2 EDTA.$2H_2O$	-	-	7.44	7.44		-
$FeCl_3$	-	-	0.65	0.65	-	-
$FeCl_3.6H_2O$	-	-	-	-	0.00056	-
$MnSO_4.4H_2O$	2.03	0.5	-	-	0.0178	0.5
$MnCl_2.4H_2O$	-	-	0.139	0.139	-	-
MoO_3	0.15	-	-	-	-	0.1
$Na_2MoO_4.2H_2O$	-	0.19	-	0.126	0.00096	-
$ZnSO_4.7H_2O$ *	0.22	0.05	-	-	0.00233	0.05
$ZnCl_2$	-	-	0.335	0.335	-	-
$CuSO_4.5H_2O$	0.079	0.02	-	-	0.00104	0.02
$CuCl_2.2H_2O$	-	-	0.001	0.001	-	-
H_3BO_3	2.86	0.5	-	-	0.0248	0.05
NH_4VO_3	0.023	0.01	-	-	-	0.01
$CoCl_2.6H_2O$	-	-	0.180	0.180	0.00096	-
$Co(NO_3)_2.6H_2O$	0.05	0.01	-	-	-	0.01
$NiSO_4.6H_2O$	0.045	0.01	-	-	0.00112	-
$Cr_2(SO_4)_3K_2SO_4.24H_2O$	0.06	0.01	-	-	0.0004	0.01
$Na_2WO_4.2H_2O$	0.018	-	-	-	0.00024	0.01
$TiO(C_2O_4K)_x.yH_2O$		-	-	-	-	0.01
$VoSO_4.2H_2O$	-	-	-	-	0.00016	-
$Al_2(SO_4)_3K_2SO_4.2H_2O$	-	-	-	-	0.00376	-

* Allen & Arnon (1955) quoted $ZnSO_4.4H_2O$, which was a misprint

Most of the experimental work was concerned with the effect of combined nitrogen on growth and morphology (Section 4.2 and 4.3). Details of the nitrogen sources and media used, are given below.

(i) Ammonium-nitrogen

NH_4Cl was used as the source of NH_4-N. As a precaution against evaporation of ammonia during autoclaving and reabsorption on cooling, stock solutions for each of the NH_4-N levels were autoclaved separately. A check was made on the level of nitrogen in the medium, before and after autoclaving (Fig. 2.1). The original media contained 0-100 mg l^{-1} NH_4Cl, which is equivalent to 0-26 mg l^{-1} NH_4Cl-N. The level of NH_4-N in the media changed during autoclaving, and hence the values obtained after autoclaving have been used. (Sections 5.21, 5.22).

Difficulties were found in buffering the media with high levels of NH_4Cl-N. As a result of these difficulties NO_3-N was used for most of the experimental work.

(ii) Nitrate-nitrogen

Sodium nitrate was used as a source of NO_3-N. This was used in preference to the potassium salt, as Calothrix scopulorum, one of the strains used, was a marine strain. The maximum level of nitrogen in the medium was designed to be greater than that of the alga, as follows: assuming that the dry weight of the algae was not likely to exceed 2 g l^{-1} and that 7% of the dry weight was nitrogen (Fay 1969), the algae would contain a maximum of 140 mg l^{-1}N. For experiments using NO_3-N (Section 5.3), the + N medium contained 140 mg l^{-1}N.

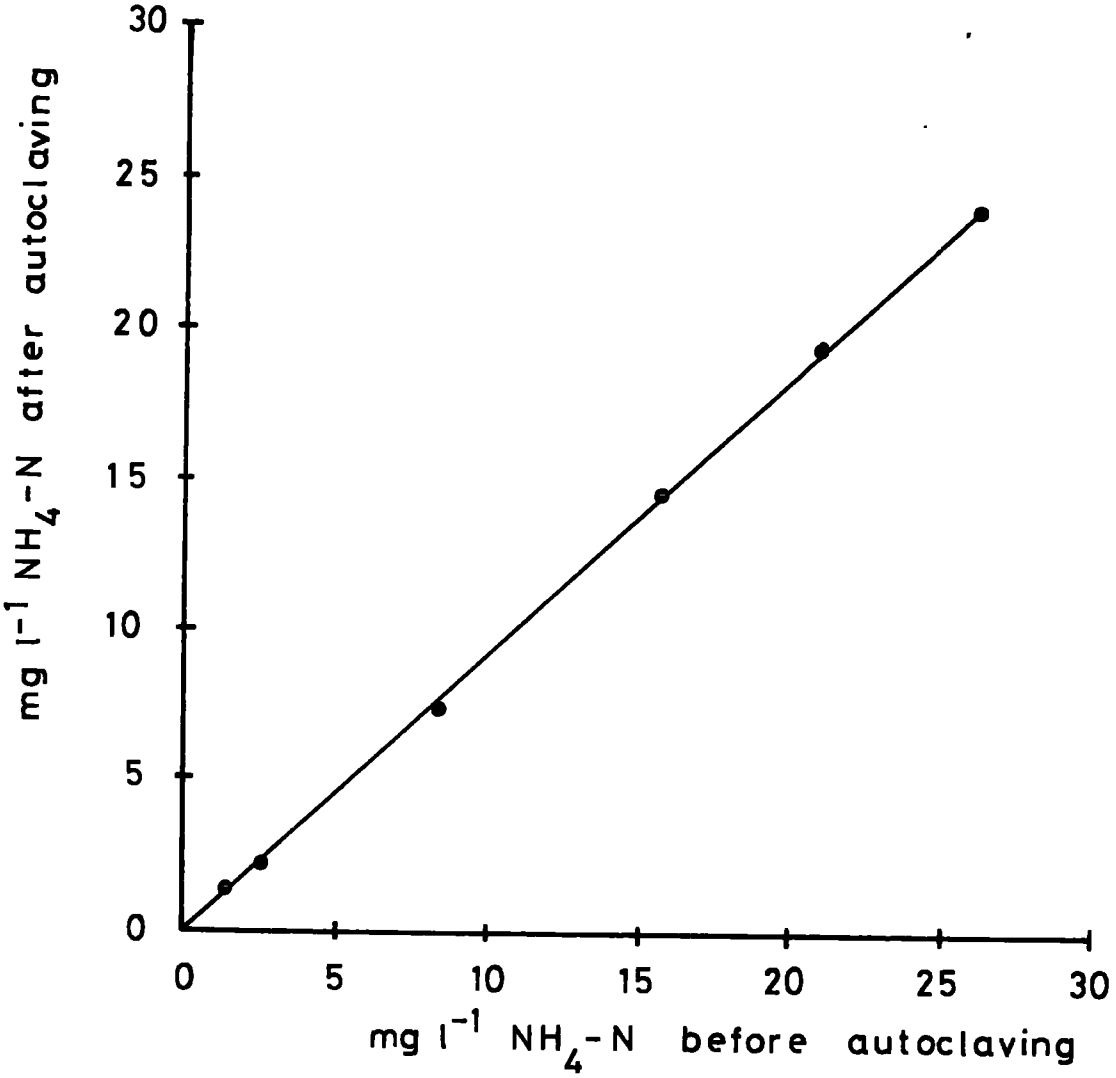


Fig. 2.1 Change in the level of NH_4-N during autoclaving

As a check that changes in the growth and morphology of the algae were not due to changes in the ionic concentration, the strains of Calothrix were also cultured in AD with additional sodium chloride. The level of sodium in this control was equal to the level of sodium in the nitrate salt (0.23 g l^{-1} Na).

2.6 Culture techniques

2.61 Cleaning of glassware

Conical flasks used for culturing the algae were washed by soaking in a hot solution of 'Quadralene' laboratory detergent. After soaking, the flasks were scrubbed to remove any algal material, rinsed thoroughly in distilled water and dried in an oven at 105°C.

If the algal material was attached too firmly to be removed by this method, the flasks were soaked overnight in an acid mixture of one volume of saturated sodium nitrite to six volumes of concentrated sulphuric acid. The flasks were then rinsed and dried as described. In order to keep the culture flasks free from nitrogen containing chemicals, this technique was used as little as possible.

Pipettes used for making up the growth media were cleaned by soaking thoroughly and washing with distilled water. Those which had been used for insoluble compounds were cleaned by soaking in HCl and then thoroughly rinsing in distilled water. Pipettes which had been in contact with organic matter were acid washed in the same way as the flasks.

2.62 Sterilisation

All liquid media are sterilised by autoclaving at 15 lb $inch^{-2}$ (103.35 Nm^{-2}) for 15 min. All solid media were sterilised by autoclaving at 10 lb $inch^{-2}$ (68.95 Nm^{-2}) for 10 min. Pipettes were plugged with non-absorbent cotton wool and sterilised in the same way as liquid media. Organic phosphate was sterilised by membrane filtration (0.22 μm Millipore filter). The parts of the filtration apparatus were wrapped in aluminium foil and sterilised in the same way as the liquid media.

Inoculation was carried out in a room which was partially sterilised prior to use. The room was prepared by spraying with absolute ethanol, to remove suspended material from the atmosphere and by irradiating with ultra-violet light for 20 min. Immediately before and after any inoculation, the bench surface was flamed. During inoculation, the necks of the flasks were heated in a Bunsen flame, in order that upward convection currents would reduce the risk of air-borne contaminants falling into the culture vessels.

2.63 Standard growth conditions

When the algae were first received from the Culture Collections, they were kept at a low light intensity of approximately 1000 lux. After several days the material was transferred to AD and ASM -1 - N (Gorham, McLachlan, Hammer and Kim 1964). The material was grown at 2000 lux and at 20°, 25° and 35°C. The optimum growth conditions were determined by inspecting three flasks of inoculum, and subsequently each strain was subcultured under these conditions (Table 2.3).

Table 2.3 Optimum growth conditions of cultures

Strain	Medium	Light Intensity (lux)	Temperature °C
Calothrix brevissima	AD	2000	25
C. desertica	AD	2000	25
C. elenkinii	AD	2000	25
C. fusca	AD	2000	25
C. gracilis	AD	2000	25
C. scopulorum	AD	2500	25
C. thermalis	AD	2000	25
C. viguieri	AD	2000	25
Calothrix sp. D 255	AD	1750	25
Calothrix sp. D 258	AD	2000	25
Calothrix sp. D 267	AD	2000	25

2.64 Inoculum material

Inoculum material for experiments was taken from cultures which were between 21-28 days old, and which had been grown in 50 ml of medium under the standard growth conditions (Table 2.3).

As many of the experimental organisms grew as a continuous mat on the surface of the liquid media, some difficulty was found in obtaining a uniform inoculum. Using a 'Whirlimix' to break up the material prior to inoculation was partially successful, but the pieces of mat were not of uniform size. Using material in the culture medium beneath the mat produced a more uniform inoculum, but the relatively small size of the inoculum resulted in very slow growth.

For experimental purposes, 1 ml of culture with pieces of algal mat of approximately the same size, was inoculated into 25 ml of media. It was necessary to use a large number of flasks as sources of inoculum, in order to be able to select pieces of approximately the same size. Furthermore, as many replicate flasks as possible were used for each experiment.

2.7 Measurement of growth and morphological variation

2.71 Dry weight

The culture was removed from the growth flask, transferred to a volumetric cylinder and the volume recorded. The culture was centrifuged at 4000 x g for 10 min. The liquid was decanted off and kept for further analysis or discarded. The algal material was then washed with distilled water and re-centrifuged at 4000 x g for 10 min. Washing and centrifuging was repeated twice. The distilled

water was decanted off and the algal material transferred to a vitreosil crucible (previously dried at 105°C). The material was dried for 36 h at 105°C.

2.72 Trichome dimensions and morphological variation

Measurements (i) and (ii) were made on trichomes from cultures which were 21 days old and had been grown under standard growth conditions (Table 2.3).

(i) The mean width and length of basal and apical cells were based on measurements of 30 trichomes. 10 trichomes were measured at random, from each of three flasks. The trichome length of each of the 30 trichomes was also recorded.

(ii) Measurements of every cell of individual trichomes were made on three trichomes only. The three trichomes of each strain were chosen at random, with the proviso that the trichomes were tapered and that every cell could be seen clearly.

(iii) The percentage of trichomes which tapered (Sections 4.21, 4.31, 4.32) was estimated by counting the number of filaments which tapered and the number which did not taper, in three fields of view, selected at random from each of three samples of algae. Three samples were taken from each of three replicate flasks. The percentage of trichomes with heterocysts was estimated in a similar way.

(iv) Estimates of trichome length and hair length, of trichomes cultured at different levels of phosphate, were based on 30 measurements taken at random from each of two algal colonies (Section 4.4).

(v) Volume and external surface area were calculated using the formulae:

$$V = \pi r^2 h$$

$$SA = 2\pi rh$$

where

V = cell volume

SA = external surface area of cell

r = $\frac{1}{2}$ cell width, measured at mid-point of cell

h = cell length

2.8 Chemical analyses

The level of NH_4-N in the experimental media (Section 4.2) was determined using the direct Nesslerisation technique (American Public Health Association, 1971).

Water samples collected in the field were immediately filtered through a number 1 'Sinta' glass funnel and later through a number 4 funnel. Samples were stored in Pyrex bottles and analysed as soon as possible after collection. The methods recommended by the American Public Health Association (1971) were used for all analyses of Si (heteropoly blue method) and of Cl (argentometric titration). All cations of samples collected since 1972 were determined by using a PERKIN ELMER atomic absorption spectrophotometer (Model 403). The levels of Na, K, Mg and Ca, in samples collected prior to this were determined by using flame photometric methods (for Na and K) and the EDTA titrimetric method (for Ca and Mg) (American Public Health Association 1965).

2.9 Acetylene reduction

Experimental procedures concerning the use of acetylene reduction technique as an assay of nitrogen fixation have been discussed by Stewart, Fitzgerald and Burris (1968). More recently the application of the acetylene reduction assay for measurement of nitrogen fixation has been discussed by Hardy, Burris and Holsten (1973).

7 ml serum bottles containing 1 ml of culture were used. 1.4 ml of acetylene was injected into each serum bottle. The algae were incubated for certain lengths of time and the amount of ethylene produced by acetylene reduction determined. (Sections 4.22, 4.31, 4.32). The gas was analysed using a gas chromatograph (VARIAN 1200 model), at an injection temperature of 70°C and a flame temperature of 100°C.

2.10 Collection of field data

Water samples and algal material were collected from several areas of Britain (Section 6.3). Each stream studied was divided into a number of reaches, as suggested by Whitton, Diaz and Hughes (1974). Each reach was approximately 10 m long but this could be reduced to allow for any changes in water chemistry or flow of water passing a theoretical transect. The number of samples collected from each reach depended on the range of microhabitats within that particular reach. A sample consisted of 3-6 colonies, which were collected for microscopic examination.

The physiognomic form of each algal sample and a description of the microhabitat was recorded according to a standard procedure being used in the Botany Department at Durham (see below). Each of the three colonies was examined microscopically to obtain an overall impression of the material e.g. the frequency of tapered filaments and parallel filaments. 10 trichomes from each preparation were taken at random and scored. However as it was necessary to make every observation on a single filament, it was often necessary to discount some of the filaments for coding purposes. Information about each filament was recorded using a standard procedure developed by the author (Section 5.2). Each filament was then identified using a program 'IDENTIFY', as described in Section 5.412.

The recording procedure used for physiognomic and environmental data incorporated the following information: sampling date, grid reference, sample number, habitat category, physiognomic category, proportion of stream bed mosaic contributed by that physiognomic category, thickness scale, substrate, substrate size, substrate microtopography, surface inclination, surface aspect, water depth at position of sample, velocity of flow at time and position of sample and exposure to light at sample point. This data was recorded as part of the program 'FIELD' and a description of the input format is given with a description of this program (Section 2.11). The coding scheme used for physiognomic and environmental factors was as follows:

Habitat category

0. Not known
1. Flowing water
2. Pond or lake
3. Terrestial

Physiognomic categories

0. Not known
1. Film
2. Filaments or filamentous floc, ± horizontal
3. Filaments, predominantly vertical
4. Totally encrusting
5. Partially encrusting
6. "Felt": interwoven filaments or other small thalli (± prostrate, without soft mucilage, often lifting as sheet)
7. Attached subspherical or hemispherical colonies, without obvious surface film
8. Attached subspherical or hemispherical colonies, with obvious surface film or other closely associated surface growth
9. (Not applicable)
10. Larger gelatinous colonies, ± orientated in direction of flow
11. Various larger plant growths, ± orientated in direction of flow
12. Various larger plant growths, ± vertical
13. "Loose" colonies, greater than 4 mm diameter
14. Algae dispersed among non-living particles
15. Planktonic, whole "standing crop"
16. Unattached, obviously deposited by stream

41. Not "standing crop", only subsample of data recorded elsewhere:
42. Not "standing crop", floating at or near surface, data not recorded elsewhere
43. Not "standing crop", plankton sampled c. 20 mm below surface, data not recorded elsewhere

61. "Aufwuchs" on larger plant: film
62. "Aufwuchs" on larger plant: filaments or filamentous floc
63. "Aufwuchs" on larger plant: filaments, predominantly vertical
64. "Aufwuchs" on larger plant: partially encrusting
65. "Aufwuchs" on larger plant: "felt"
66. "Aufwuchs" on larger plant: attached subspherical or hemispherical colonies, without obvious surface film
67. "Aufwuchs" on larger plant: attached subspherical or hemispherical colonies, with obvious surface film
68. "Aufwuchs" on larger plant: larger gelatinous colonies, ± orientated in direction of flow

Categories 7 and 8 are particularly relevant to *Rivularia* colonies

B. Proportion of stream bed mosaic (living + non-living) contributed by same physiognomic forms as the sample unit

0. Not known
1. 0 - 0.1%
2. 0.1 - 1%
3. 1 - 10%
4. 10 - 100%
5.
6.
7.
8.
9. (Not applicable)

C. Thickness scale

0. Not known
1. Very thin
2. Thin
3. Moderate +

9. Not applicable

D. Substrate

0. Not known
1. Metamorphosed limestone
2. Magnesian limestone
3. Other limestone
4. Chalk
5. Sandstone (coarse or medium), without lime
6. Igneous rocks, or compacted sandstone, without lime
7. Sandstone (coarse or medium), with detectable lime
8. Igneous rocks, or compacted sandstone, with detectable lime
9. Not applicable, including 'obviously mixed'
10. Shale
11. Cement
12. Brick
13. Wood
14. Peat
15. Plastic

E. Substrate size

0. Not known
1. Sheet
2. Large boulder
3. Medium boulder
4. Large pebble or small boulder
5. Small pebble
6. Sand-size particles
7. Coarse or medium silt
8. Very fine silt
9. Not applicable

F. Substrate microtopography

0. Not known
1. Emergent above average level
2. Plane
3. Hollows (± round)
4. Crack across flow
5. Crack with flow
6. Channel (less than 50 mm wide, and at least 30 mm deep)
7.
8.
9. Not applicable

G. Surface inclination

0. Not known
1. None detectable visually
2. Just detectable visually 10^{o}
3. $10\text{-}45^{o}$
4. $45\text{-}85^{o}$
5. Approximately vertical
6. More than vertical
7. Lip
8.
9. Not applicable

H. Surface aspect

0. Not known
1. Upstream
2. Downstream
3. Side
4. Underneath, or nearly so
5.

9. Not applicable

I. Water depth at position of sample

0. Not known
1. Dry
2. Moist, or very thin film (< 2 mm)
3. 2 - 20 mm
4. 20 - 100 mm
5. Over 100 mm

9. Not applicable

J. Rate of flow at time and point of sampling
(in relation to that of main current on that particular time)

0. Not known
1. None
2. Slow
3. Medium
4. Fast
5. Very fast

9. Not applicable

K. Exposure to light at actual sample point

0. Not known
1. More or less open through year
2. Moderate cover through year
3. Heavy shade through year
4. Moderate shade in summer
5. Heavy shade in summer
6.
7.
8.
9. Not applicable

2.11 Computing

All computing was done using the Durham link to the IBM 360 at Newcastle. Three programs were written for this work. Descriptions of their use and instructions concerning data input are given below.

(i) FIELD

This program was designed to record details of microhabitat, physiognamy and trichome morphology. Microhabitat and physiognomic data were coded as described above, trichome data was coded according to a scheme outlined in Section 5.2. The program was designed to record data in a table form, for ease of presentation and in a form in which it could be used with two other programs 'IDENTIFY' and 'QUESTION'. The program is listed in Table 2.4. The data was punched in the following format:

Card 1, habitat data which is punched as:

Col. 1-6, a six digit integer representing the date e.g. 161071 represents a 16th. October 1971

Col. 8-15, an alphanumeric of 2 letters and 6 integers representing the grid reference

Col. 17-21, a five digit integer representing the sample number

Col. 23-24, a two digit integer representing the habitat category

Col. 26-27, a two digit integer representing the physiognomic category

Col. 29, 31, 33, 35, 37, 39, 40, 41, 43, 45, 47, eleven separate integers representing the codes for habitat categories B-K, as described in the recording scheme (p. 57)

Card 2, macrocharacter data which is punched as:

Col. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, twelve separate integers representing the macrocharacters 1-12, as described in Section 5.23 (for computational purposes these were designated A1-L1)

Subsequent cards, trichome data. The data for one trichome is punched on one card as:

Col. 1-2, trichome number

Col. 4, an integer representing trichome character A2 (Characters A2 - V2 are described in Section 5.23)

Col. 6, an integer representing trichome character B2

Col. 8-11, and 13-16, two, four digit integers representing the trichome characters C2 and D2

Col. 18-21, a real number representing trichome character E2

Col. 23, 24, 27, 29, 31, 33, 35, seven separate integers representing trichome characters F2 - L2

Col. 37-40 and 42-45, two real numbers representing trichome characters M2 and N2

Col. 47 and 49, two integers representing trichome characters O2 and P2

Col. 51-54, a real number representing trichome character Q2

Col. 56, 58, 60, 62, 64, five integers representing trichomes characters R2 - V2

Samples are separated by a card with 00 punched in columns 1 and 2. The end of the data is indicated by a card punched as 000-1 in the first five columns.

(ii) 'IDENTIFY'

This program was designed to identify unknown species by comparing the unknown in turn with each known species (Section 5.411). The species showing the closest agreement with the unknown is taken as the correct identification. The coefficients of resemblance used with this program are described in Section 5.41. Data were coded according to the scheme given in Section 5.2 and input in F2 format i.e. an integer up to two digits long. (If there is only one digit it is right justified). A listing of the program is given in Table 2.5.

(iii) 'QUESTION'

This program was designed to answer data queries, using the information collected from the floras, particularly from Geitler (1932). Data (Appendix II) were coded in the way described for 'IDENTIFY' (above). A listing of the program is given in Table 2.6.

2.12 Statistics

Means and standard errors were calculated using a computer program produced by Durham University Computer Department. Comparisons of results using the standard error of the difference

between the means and the t-test were based on Morony (1951). Water chemistry data (Section 6.3) was compared using the non-parametric Mann-Whitney U-test (Siegel 1956, Elliott 1971).

Table 2.4 Copy of 'FIELD' program

```
C TO PRINT RIVULARIA RECORDS IN TABLE FORM
      REAL *8 GRF,GI,M2,N2,O2
      INTEGER N,TN,DAT1,NO,HC,A,B,C,D,E,F,G,H,I,J,K,A1,B1,C1,D1,SUB,E1,F
     1I,G1,H1,I1,J1,K1,L1,A2,B2,C2,D2,E2,F2,H2,I2,J2,K2,L2,P2,Q2,R2,S2
C INITIALISE SERIAL NUMBER
      N=10001
C READ HABITAT DATA
 600      WRITE(6,500)
 500      FORMAT(1H1////////////)
    9 READ(5,2) DAT1,GRF,NO,HC,A,B,C,D,E,F,G,H,I,J,K
    2 FORMAT(I6,1X,A8,1X,I5,1X,I2,1X,I2,10I2)
      IF(DAT1.LT.0) GO TO 12
C WRITE HABITAT HEADING AND DATA
      WRITE(6,3)
  3           FORMAT(////16X,
     1                    58H  DATE  GRIDREF   NUM  HC  A  B  C  D  E  F
     2 G  H  I  J  K)
      WRITE(6,4)DAT1,GRF,NO,HC,A,B,C,D,E,F,G,H,I,J,K
  4     FORMAT(17X,
     1           I6,1X,A8,1X,I5,1X,I2,11I3)
C WRITE MACRC HEADING
      WRITE(6,5)
    5 FORMAT(1HO,17X,
     1               15HMACRCCHARACTERS,8X,23HA B C D E F G H I J K L)
C READ AND WRITE MACRC DATA
      READ(5,6) A1,B1,C1,D1,E1,F1,G1,H1,I1,J1,K1,L1
    6 FORMAT(12I2)
      WRITE(6,7) A1,B1,C1,D1,E1,F1,G1,H1,I1,J1,K1,L1
    7 FORMAT(1HO,39X,12I2)
C WRITE TRICHOME HEADING
      WRITE(6,13)
 13     FORMAT(1HO,16X,9HTRICHOMES/17X,
     177HTN 1 2    3    4    5    6 7 8 9 10 11 12 13   14   15 16 17
     218 19 20 21 22)
   11 READ(5,8)TN,A2,B2,C2,D2,E2,F2,G2,H2,I2,J2,K2,L2,M2,N2,O2,P2,Q2,R2,
```

```
     1 S2,T2,U2,V2
     8 FORMAT(3I2,2I5,F5.1,7I2,2F5.1,2I2,F5.1,5I2)
       IF(TN.EQ.0)GO TO 14
C WRITE TRICHOME DATA
       WRITE(6,10)TN,A2,B2,C2,D2,E2,F2,G2,H2,I2,J2,K2,L2,M2,N2,O2,P2,Q2,R
      12,S2,T2,U2,V2
    10 FORMAT(1HC,16X,
      1            3I2,2I5,1X,F5.1,2X,4I2,3I3,2F5.1,2I3,F5.1,5I3)
       GO TO 11
    14 N=N+1
       GOTO 600
    12 STOP
       END
```

Table 2.5 Copy of 'IDENTIFY' program

```
 IDENTIFY: PROC OPTIONS(MAIN);

/*       IDENTIFY PROGRAM

    P L / 1

PROGRAM TO IDENTIFY UNKNOWN SPECIES BY COMPARING THE UNKNOWN, IN TURN,
WITH EACH KNOWN SPECIES. THE SPECIES SHOWING THE CLOSEST AGREEMENT WITH THE
UNKNOWN IS TAKEN AS THE CORRECT IDENTIFICATION.

SEVERAL COEFFICIENTS OF RESEMBLANCE CAN BE USED WITH THIS PROGRAM
(SECTION 6.32)

DATA IMPUT:
CARD 1:TWO  INTEGERS LOCATED ANYWHERE ON THE CARD, INDICATING THE
NUMBER OF KNOWN SPECIES (OR DATA SETS) AND THE NUMBER OF VARIABLES
PRESENT FOR EACH SPECIES.
SUBSEQUENT CARDS: DATA FOR EACH SPECIES IN THE FORM:-
FIRST CARD, THE SPECIES NAME, UP TO 80 CHARACTERS LONG.
SECOND OR FOLLOWING CARDS, THE RESPONSES FOR THE VARIABLES.
THE DATA FOR THE NEXT SPECIES FOLLOWS. THERE IS NO NEED TO INDICATE THE
END OF THE DATA. A MAXIMUM OF 1000 SPECIES IS ALLOWED FOR. THE DATA ARE
LISTED AS INPUT. THE VARIABLES ARE STORED IN A TWO DIMENSIONAL ARRAY A.


PROGRAM
                                                                      */
GET LIST(NS,NV);
/* NS = NO. OF KNOWN SPECIES , NV = NO. OF VARIABLES PER SPECIES */
BEGIN;
DCL NA CHAR(80);
DCL D(NS,NV) FIXED BIN,U(NV) FIXED BIN, S(NS) FLOAT;
DCL NAM(NS) CHAR(80);
DO I = 1 TO NS;
GET EDIT(NAM(I))(COL(1),A(80));
```

```
PUT EDIT(NAM(I))(COL(10),A);
GET EDIT((D(I,J) DO J=1 TO NV))
(COL(1),4 F(2),F(2),F(2),F(2),4 F(2),F(2),5 F(2),F(2),F(2),F(2),6 F(2))
;
PUT EDIT((D(I,J) DO J = 1 TO NV))(COL(4),27 F(2));
END;
/* READ IN UNKNOWN AND COMPARE WITH KNOWN SPECIES */
PUT EDIT('UNKNOWN')(COL(20),A);
PUT SKIP;
AGAIN: GET EDIT(NA)(COL(1),A(80));
PUT EDIT(NA)(COL(10),A);
GET EDIT((U(J) DO J=1 TO NV))
(COL(1),4 F(2),F(2),F(2),F(2),4 F(2),F(2),5 F(2),F(2),F(2),F(2),6 F(2))
;
PUT EDIT((U(I) DO I = 1 TO NV))(COL(4),27 F(2));
PUT SKIP(5);
DMAX = 0.0; DUS =0.0;
NOTAP=0;
DO I = 1 TO NS;
DO J = 1 TO NV;
IF U(J)=D(I,J)THEN GO TO SUE;
IF D(I,J)=0|D(I,J)=9 THEN GO TO SUE;
IF U(J)=0|U(J)=9 THEN GO TO SUE;
NOTAP=NOTAP+1;
SUE:
IF D(I,J)=U(J)&D(I,J)>0.0&D(I,J)¬=9.0 THEN DUS=DUS+1;
END;
S(I)=DUS/(DUS+NOTAP);
NOTAP=0;
DUS=0.0;
END;
PUT EDIT('NEAREST SPECIES')(COL(10),A);
PUT SKIP;
DMAX=-100.0;
DO I = 1 TO NS;
```

```
IF S(I)> DMAX THEN DMAX=S(I);
END;
DO I = 1 TO NS;
S(I)=S(I)-DMAX;
IF S(I)>= 0.0 THEN DO;
PUT EDIT(NAM(I))(COL(30),A);
PUT EDIT((D(I,J) DO J = 1 TO NV))(COL(4),27 F(2));
END; END;
GOTO AGAIN;
END: END; END;
```

Table 2.6 Copy of 'QUESTION' program

```
QUEST: PROC OPTIONS(MAIN);

/*       QUERY  PROGRAM

      P L / 1

PROGRAM TO LIST THOSE DATA SETS FOR WHICH A PARTICULAR QUERY IS TRUE.
DATA INPUT :
CARD 1: AN INTEGER, LOCATED ANYWHERE ON THE CARD, INDICATING THE NUMBER OF
VARIABLES PRESENT FOR EACH SPECIES
SUBSEQUENT CARDS: DATA FOR EACH SPECIES IN THE FORM:-
FIRST CARD, THE SPECIES NAME, UP TO 80 CHARACTERS LONG.
SECOND OR FOLLOWING CARDS, THE RESPONSES FOR THE VARIABLES.
THE DATA FOR THE NEXT SPECIES FOLLOWS. THERE IS NO NEED TO INDICATE THE
END OF THE DATA. A MAXIMUM OF 1000 SPECIES IS ALLOWED FOR. THE DATA ARE
LISTED AS INPUT. THE VARIABLES ARE STORED IN A TWO DIMENSIONAL ARRAY A.

 THE QUERY IS SET AS A PL/1 IF STATEMENT, FOR EXAMPLE,

IF A(I,10)=1 | A(I,10)=2 | A(I,10)=3 THEN DO;

SETS THE QUERY 'DOES THE SPECIES HAVE A HAIR(VARIABLE NUMBER 10)?'.
A POSITIVE ANSWER IS GIVEN WHETHER THE HAIR IS LONG (CODE1), SHORT
(CODE2) OR PRESENT (CODE 3).
EACH QUERY IS INCLUDED IN A DO LOOP. TWO EXAMPLE QUERIES ARE INCLUDED. */


PROGRAM

GET LIST(NV);
BEGIN;
DCL NAM(1000) CHAR(80);
DCL A(1000,NV) FLOAT;
ON ENDFILE(SCARDS) GOTO FIN;
K=1;
```

```
DO I = 1 TO 1000;
GET EDIT(NAM(I))(COL(1),A(80));
PUT EDIT(NAM(I))(COL(10),A);
GET EDIT((A(I,J) DO J = 1 TO NV))(COL(1),27 F(2));
PUT EDIT((A(I,J) DO J = 1 TO NV))(COL(4),27 F(2));
K=K+1; END;
FIN: K=K-1;
PUT EDIT('--------------------------')(COL(5),A);
DO I = 1 TO K;
/* ENTER QUERY HERE */
IF A(I,10)=1&A(I,22)=1|A(I,10)=2&A(I,22)=1|A(I,10)=3&A(I,22)=1
THEN DO;
PUT EDIT(NAM(I))(COL(20),A);
PUT EDIT((A(I,J) DO J = 1 TO NV))(COL(4),27 F(2));
END; END;
PUT EDIT('--------------------------')(COL(5),A);
DO I = 1 TO K;
IF A(I,10)=1&A(I,22)=4|A(I,10)=2&A(I,22)=4|A(I,10)=3&A(I,22)=4
THEN DO;
PUT EDIT(NAM(I))(COL(20),A);
PUT EDIT((A(I,J) DO J = 1 TO NV))(COL(4),27 F(2));
END; END;
PUT EDIT('--------------------------')(COL(5),A);
END; END;
```

3 TAPERING AND THE HAIR

3.1 Introduction

The background literature concerning tapering and the hair has been described in Section 1.3. As this information was largely qualitative, an attempt was made to develop a more quantitative approach. It is only in this way that comparisons can be made which might provide some evidence for the factors which influence the development of tapering and hair formation.

The preliminary definition of tapering (1.3) implies a gradual reduction in the diameter of a trichome. This concept has a limited use, not only because of its qualitative nature, but also because it is an oversimplification of the actual shape of a tapered trichome. The hair has been defined as a series of elongated, colourless cells (1.32) however for comparative work a quantitative measurement is again essential.

For simplicity, and because no a priori relationship between tapering and hair development should be implied, these two characters are considered separately. The concept of tapering has been considered first, with the aim of finding a numerical way of expressing the change in shape that occurs in tapered trichomes.

During later parts of this study, work on tapering and the hair was undertaken with several other people (Whitton, Kirkby, Peat and Sinclair 1973). The results of this work have considerable significance to the results of this thesis, and are mentioned briefly in Section 3.5 and in more detail in Section 7.6.

3.2 The concept of tapering

As pointed out in Section 3.1, the preliminary definition of tapering has a relatively limited use. It only allows a simple yes/no answer to the question of whether a trichome tapers or not, and this in turn imposes three problems:

(i) It cannot be used in assessing any of the differing extents to which tapering is exhibited, under various environmental conditions.

(ii) It does not describe whether tapering of a trichome changes in any way during development, and if so how.

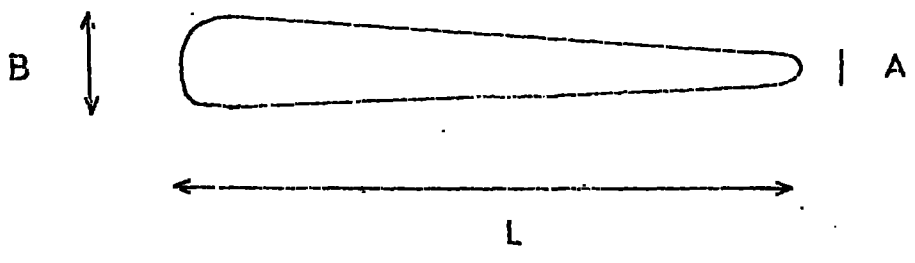
(iii) The term can not be used for comparing different organisms and their responses, except in simple positive and negative terms.

For practical reasons it would be very useful to devise a quantitative index of tapering which would allow comparisons of tapering to be made. No attempt has been made to describe the change in shape of a trichome, from the homogonial stage to a tapered form, in quantitative terms.

3.21 Indices of tapering

In its simplest form a tapered trichome may be considered as a cone. Several indices of tapering were derived from this shape (below). The notation used in deriving these indices are shown in Fig. 3.1.

Fig. 3.1 Notations used to derive indices of tapering



B = maximum trichome diameter
A = minimum trichome diameter
L = total trichome length

Based on the preliminary definition of tapering (Section 3.1), the change in the diameter of a trichome can be represented by a tapering index T_1 as follows:

$$T_1 = B - A$$

If instead of considering absolute differences, the difference is expressed as a percentage of maximum width, then a second index of tapering, T_2 can be represented by

$$T_2 = \frac{B - A}{B} \times 100$$

Using T_1 or T_2, a parallel hormogonium would produce a tapered trichome under the following conditions:

(i) The base of the trichome became swollen, but the apical width remained the same as shown in Fig. 3.2 (a) and (b).

(ii) The basal width remained the same but the apical width became narrower, as shown in Fig. 3.2 (c) and (d).

(iii)The base became wider and the apical end of the trichome narrowed, as shown in Fig. 3.2 (e) and (f).

The range in the basal and apical widths of 30 trichomes of 11 species of Calothrix and 4 other Rivulariaceae were plotted against T_1 (Fig. 3.3). These results indicate that in most cases, the basal width increases and the apical width decreases, as T_1 increases. This suggests that changes in both the basal and apical widths are responsible for changes in the taper of the trichomes. In some cases, the change in basal width was numerically greater, as in C. desertica, C. elenkinii, C. fusca, C. scopulorum, C. viguieri,

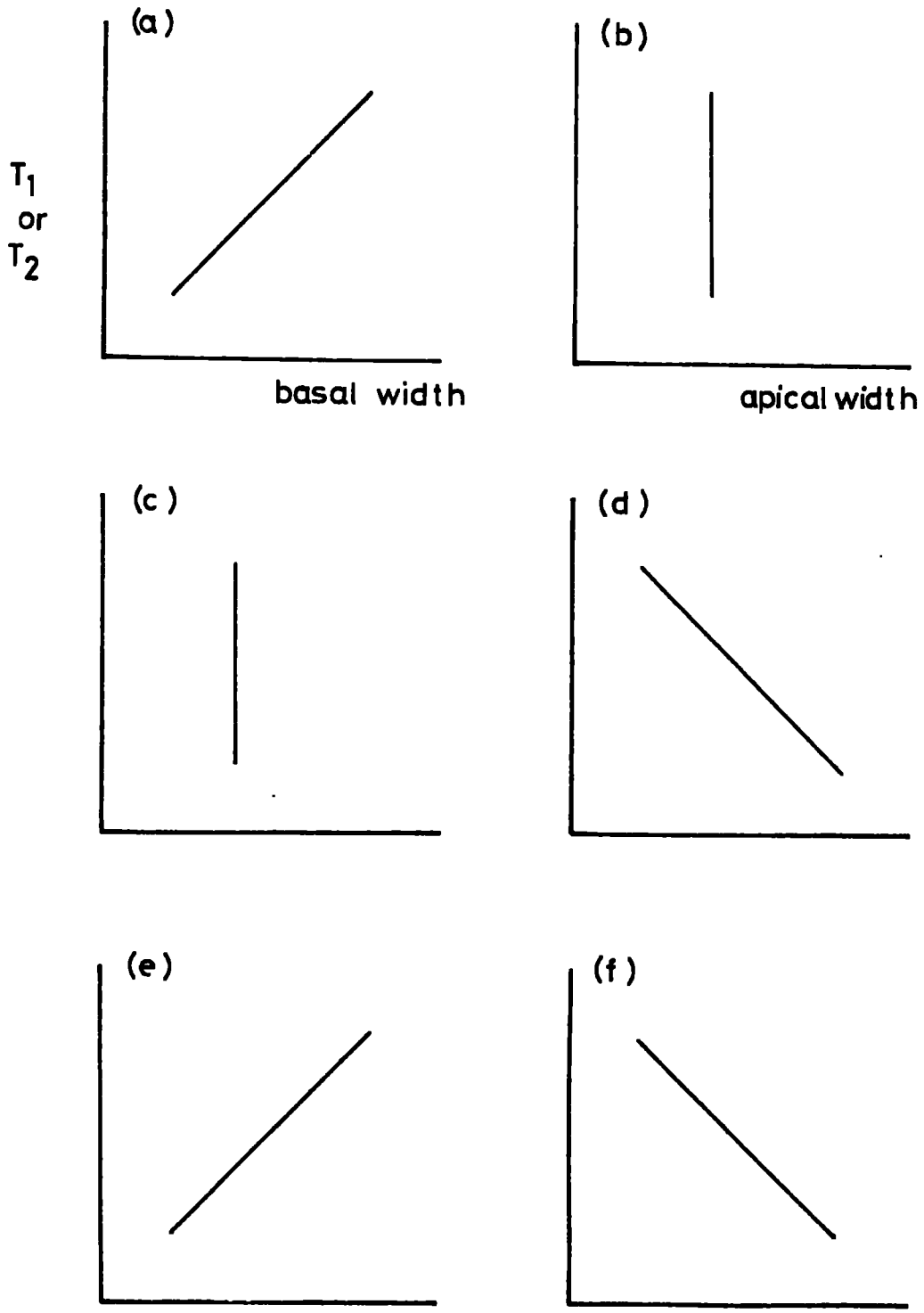
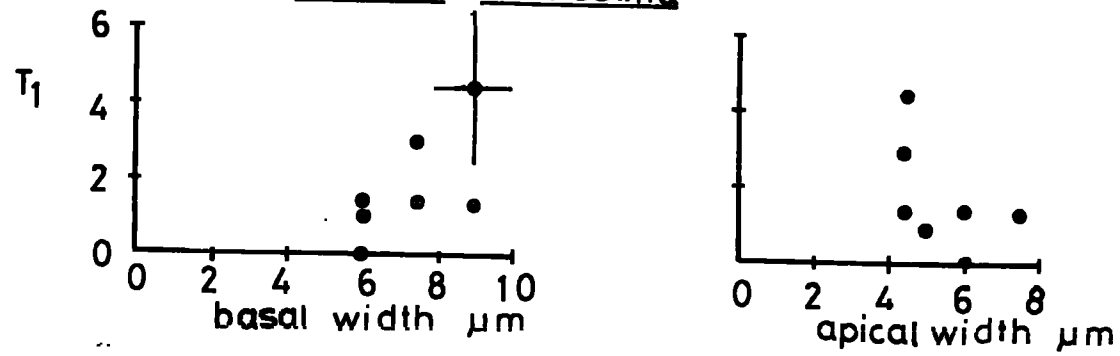


Fig. 3.2 Changes in trichome shape which produce tapering

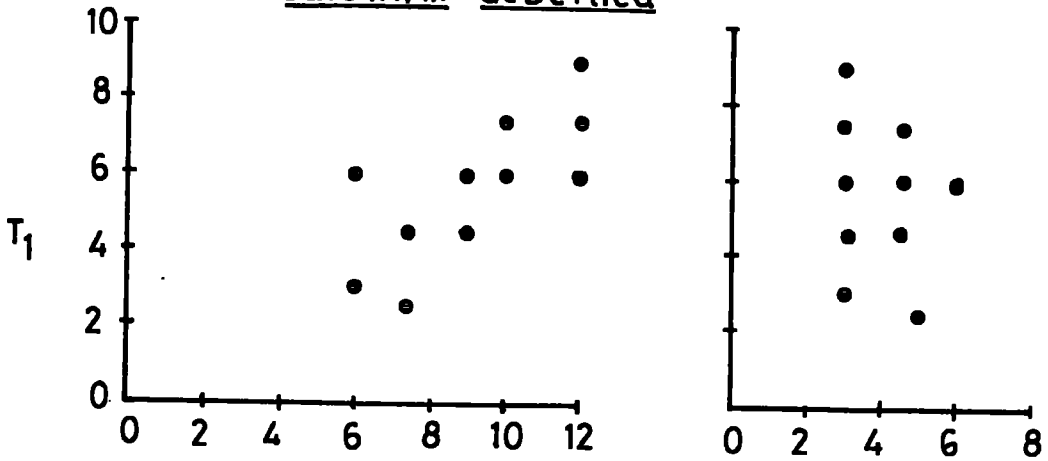
Fig. 3.3 Variation of maximum and minimum trichome widths with tapering index T_1

Results are shown for 11 species of Calothrix and 4 Rivulariaceae. (Errors of $\pm$ 1 µm were possible during measuring, error bars being indicated on the first graph only.)

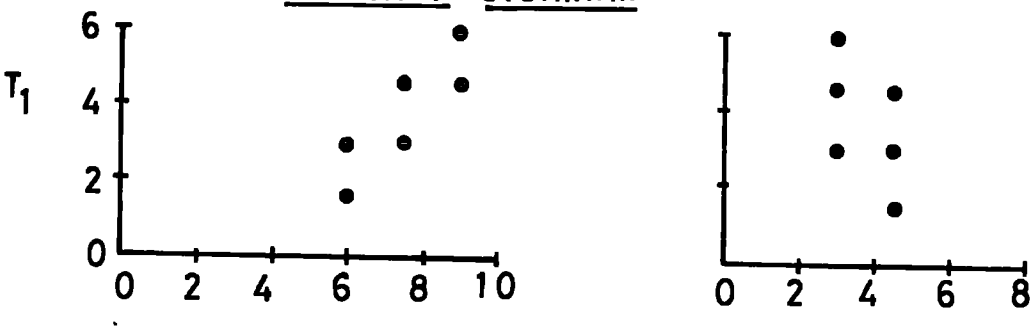
Calothrix brevissima



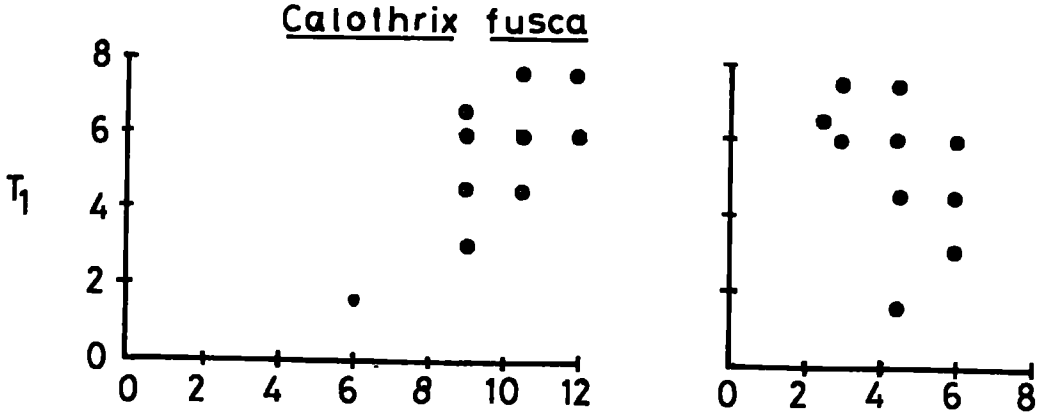
Calothrix desertica

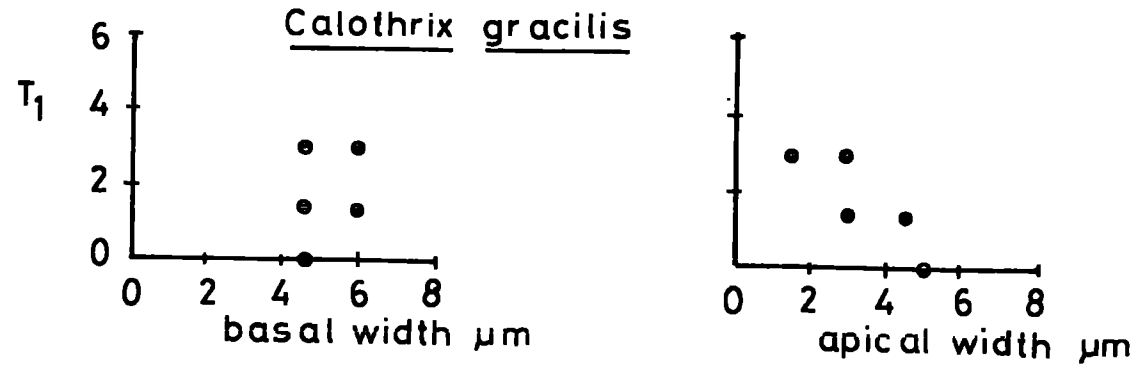


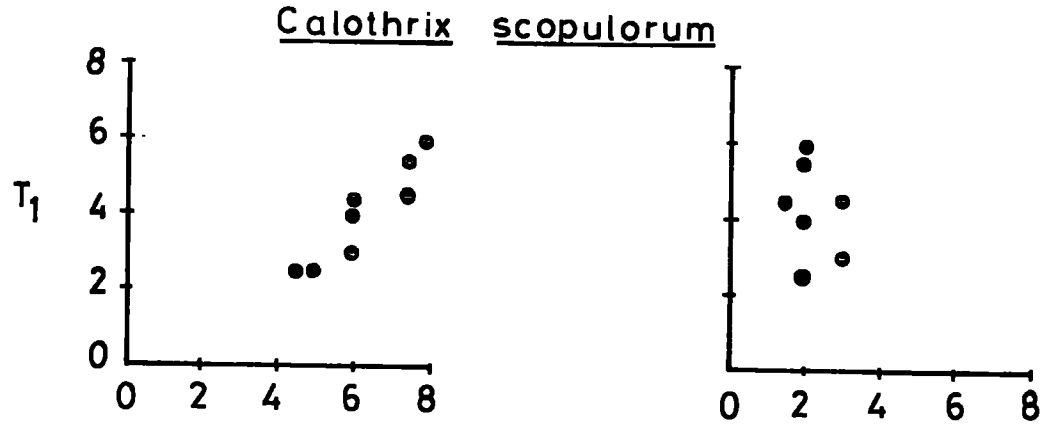
Calothrix elenkinii

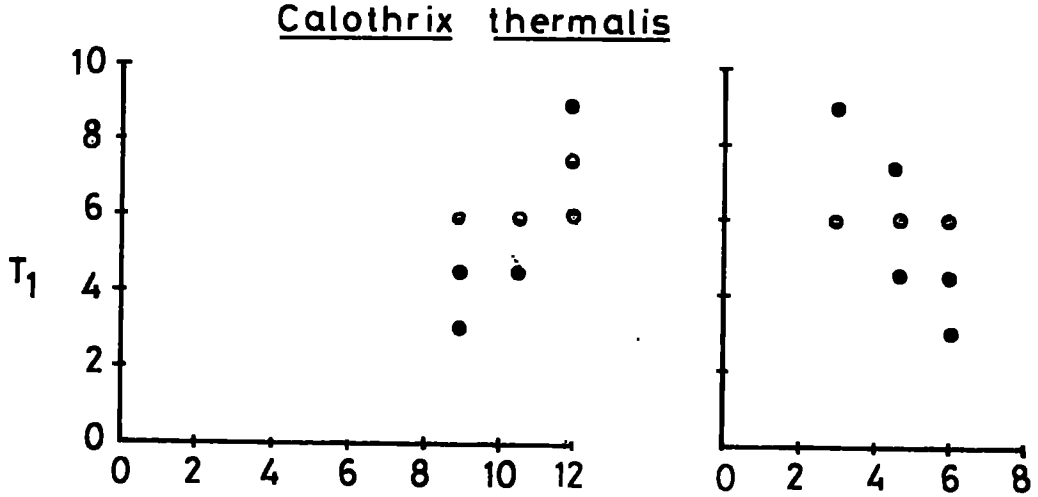


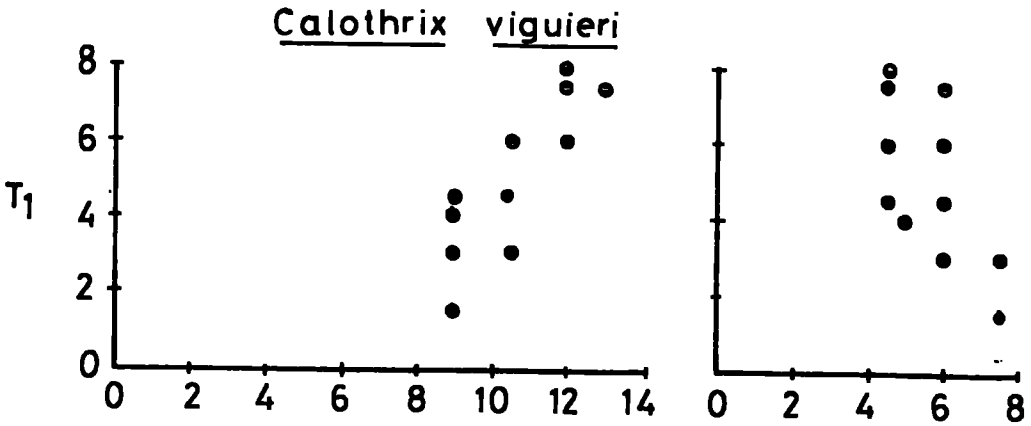
Calothrix fusca



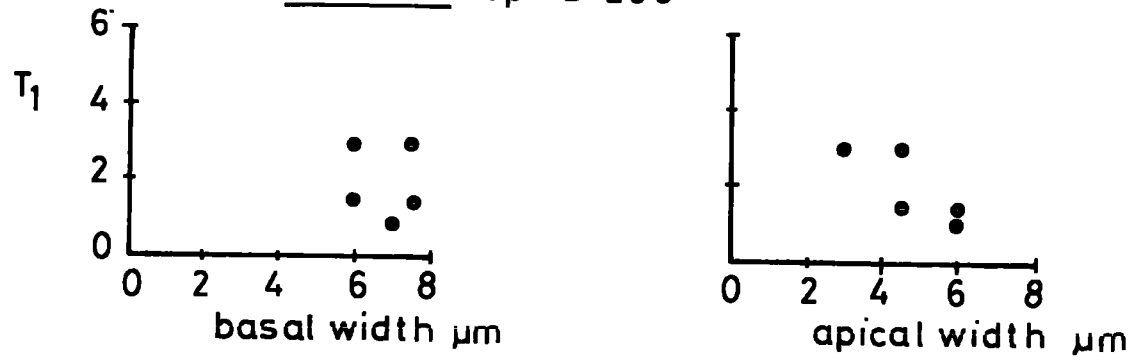

Calothrix gracilis
T1
basal width µm
apical width µm


Calothrix scopulorum
T1

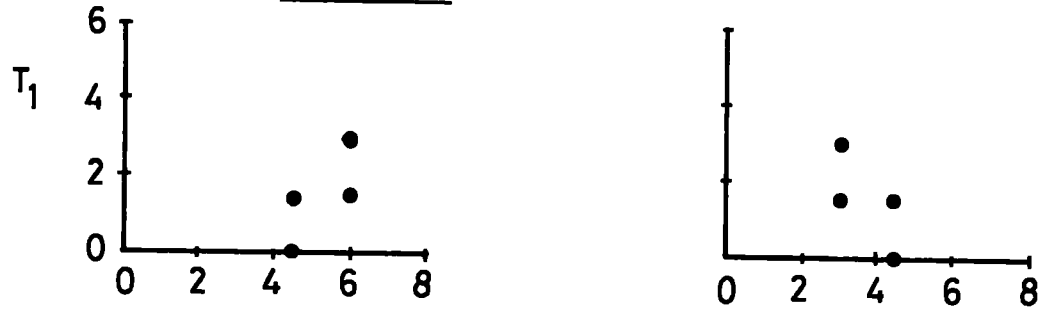

Calothrix thermalis
T1


Calothrix viguieri
T1

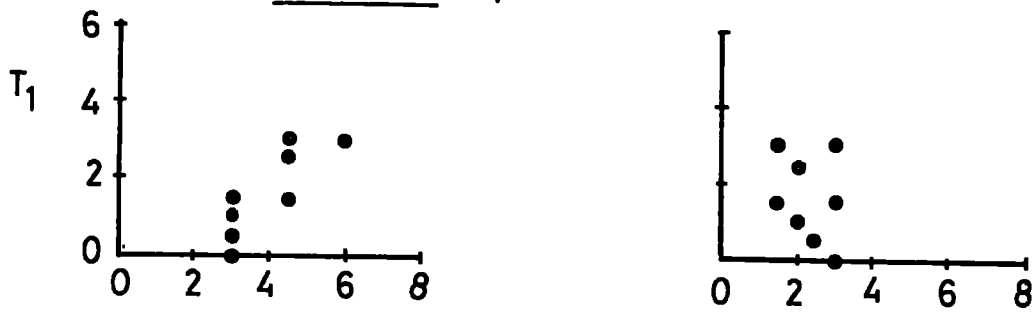
Calothrix sp. D 255



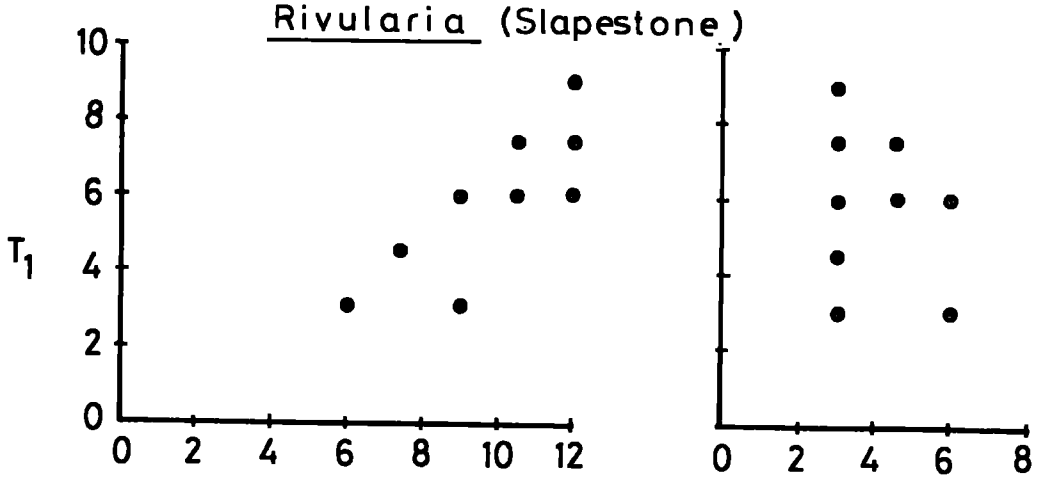
Calothrix sp. D 258

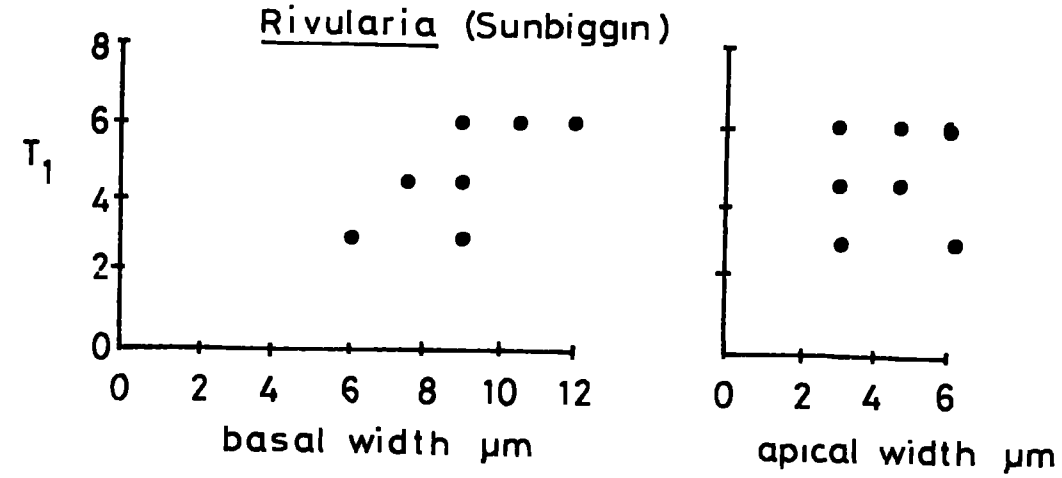


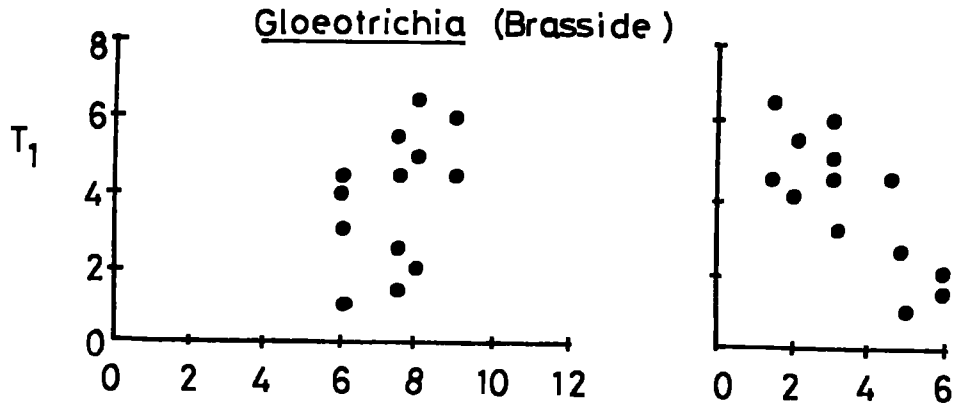
Calothrix sp. D 267

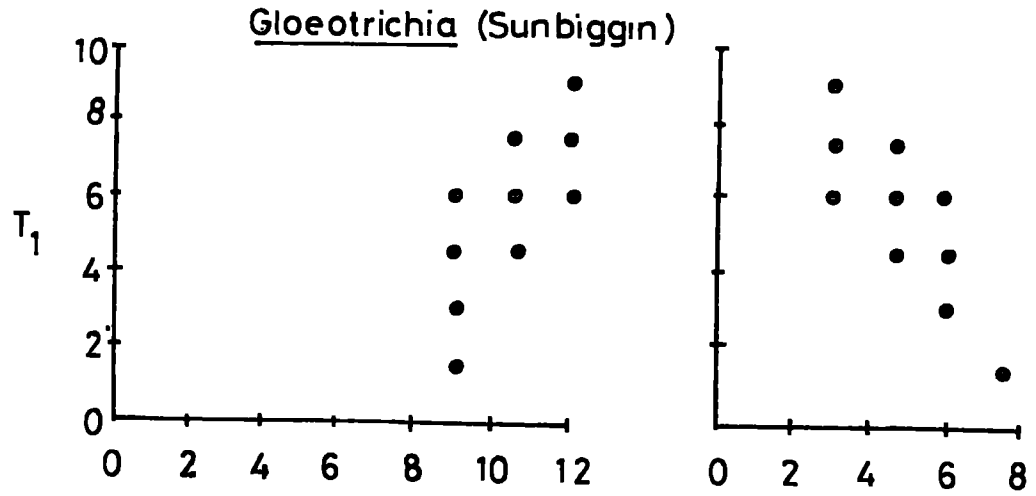


Rivularia (Slapestone)




Rivularia (Sunbiggin)
T1
basal width µm
apical width µm


Gloeotrichia (Brasside)
T1


Gloeotrichia (Sunbiggin)
T1

Calothrix sp. D 267 and two field Rivularia samples. Changes due to a decrease in width at the apical end, as T_1 increases were relatively greater in Calothrix gracilis, Calothrix sp. D 255 and two Gloeotrichia samples.

Having considered simply the change in the diameter of a trichome, it is now necessary to take into consideration the length of the trichome as well. If two trichomes have the same maximum and minimum diameters, but are of different lengths, then the shorter trichome is more tapered. This is because the rate of change in the diameter of the trichome is greater. Two further indices of tapering which include all three parameters, may be expressed as follows:

$$T_3 = \frac{B-A}{L} \times 100 = \frac{T_1}{L} \times 100$$

$$T_4 = \frac{\frac{B-A}{B} \times 100}{L} = \frac{T_2}{L} \times 100$$

The tapered appearance of 11 species of Calothrix and 4 other Rivulariaceae was studied using the four indices of tapering which have been described. The dimensions of the basal and apical cells of these species are given in Table 3.1. Based on these measurements, values for each of the four indices of tapering were calculated (Table 3.2). The species were arranged in order, beginning with the most tapered i.e. the highest value for each tapering index (Table 3.3). As might be expected the four orders are similar but not identical.

Table 3.1 Trichome dimensions (measured in μm)

Species	basal width	apical width	basal length	apical length
Calothrix brevissima	6.4 ± 0.16	5.5 ± 0.14	4.8 ± 0.13	3.7 ± 0.16
C. desertica	8.9 ± 0.14	3.4 ± 0.14	-	-
C. elenkinii	7.3 ± 0.21	3.4 ± 0.12	-	-
C. fusca	10.1 ± 0.24	4.8 ± 0.22	4.4 ± 0.14	5.9 ± 0.08
C. gracilis	4.7 ± 0.08	3.8 ± 0.16	7.2 ± 0.11	3.4 ± 0.17
C. scopulorum	6.2 ± 0.16	2.3 ± 0.13	3.3 ± 0.11	2.7 ± 0.22
C. thermalis	10.0 ± 0.23	5.0 ± 0.18	5.4 ± 0.13	3.1 ± 0.12
C. viguieri	11.0 ± 0.26	5.3 ± 0.18	7.0 ± 0.13	7.0 ± 0.19
Calothrix sp. D 255	6.2 ± 0.09	5.6 ± 0.14	6.3 ± 0.10	5.1 ± 0.13
Calothrix sp. D 258	5.3 ± 0.14	3.7 ± 0.14	3.3 ± 0.11	4.0 ± 0.13
Calothrix sp. D 267	4.4 ± 0.15	2.6 ± 0.10	5.7 ± 0.30	2.9 ± 0.05
Gloeotrichia (Brasside)	10.2 ± 0.21	4.3 ± 0.23	7.3 ± 0.11	19.4 ± 1.10
Gloeotrichia (Sunbiggin)	7.4 ± 0.18	3.2 ± 0.26	8.4 ± 0.13	10.3 ± 0.19
Rivularia (Slapestone)	10.4 ± 0.34	4.2 ± 0.25	-	-
Rivularia (Sunbiggin)	8.4 ± 0.27	3.6 ± 0.17	-	-

Table 3.2 Tapering values

Species	T_1	T_2	T_3	T_4
Calothrix brevissima	0.9 ± 0.21	12.3 ± 2.69	0.8 ± 0.17	12.0 ± 2.50
C. desertica	5.5 ± 0.26	61.6 ± 1.80	1.5 ± 0.11	19.2 ± 2.00
C. elenkinii	3.9 ± 0.21	52.1 ± 1.90	1.5 ± 0.10	21.1 ± 1.44
C. fusca	5.3 ± 0.23	53.0 ± 2.03	1.8 ± 0.11	18.5 ± 0.91
C. gracilis	0.9 ± 0.17	17.7 ± 3.47	0.3 ± 0.06	5.4 ± 1.11
C. scopulorum	3.8 ± 0.18	61.3 ± 1.79	6.3 ± 0.66	98.8 ± 8.48
C. thermalis	5.0 ± 0.25	47.0 ± 2.09	1.2 ± 0.10	11.7 ± 0.85
C. viguieri	5.6 ± 0.34	50.3 ± 2.24	2.8 ± 0.34	26.7 ± 3.52
Calothrix sp. D 255	0.6 ± 0.16	9.4 ± 2.53	0.3 ± 0.08	4.1 ± 1.10
Calothrix sp. D 258	1.6 ± 0.12	29.9 ± 2.06	0.7 ± 0.09	12.3 ± 1.65
Calothrix sp. D 267	1.7 ± 0.15	38.0 ± 2.82	2.7 ± 0.26	55.1 ± 5.63
Gloeotrichia (Brasside)	4.2 ± 0.30	56.1 ± 3.68	1.1 ± 0.60	14.7 ± 1.37
Gloeotrichia (Sunbiggin)	5.9 ± 0.32	57.4 ± 2.50	1.5 ± 0.12	14.4 ± 1.03
Rivularia (Slapestone)	6.2 ± 0.33	59.0 ± 2.31	1.2 ± 0.13	11.8 ± 1.44
Rivularia (Sunbiggin)	4.8 ± 0.22	56.9 ± 1.63	1.0 ± 0.09	11.6 ± 0.93

Table 3.3 Tapering order, beginning with the highest value of each tapered index

	T_1	T_2	T_3	T_4
1.	Rivularia (Slapestone)	C. desertica	C. scopulorum	C. scopulorum
2.	Gloeotrichia (Sunbiggin)	C. scopulorum	C. viguieri	Calothrix sp. D 267
3.	C. desertica }	Gloeotrichia (Brasside)	Calothrix sp. D 267	C. viguieri
4.	C. viguieri }	Rivularia (Slapestone)	C. fusca	C. elenkinii
5.	C. fusca	Gloeotrichia (Sunbiggin)	C. desertica	C. desertica
6.	C. thermalis	Rivularia (Sunbiggin)	C. elenkinii	C. fusca }
7.	Rivularia (Sunbiggin)	C. fusca	Gloeotrichia(Sunbiggin) }	Gloeotrichia (Brasside)
8.	Gloeotrichia (Brasside)	C. elenkinii	C. thermalis	Gloeotrichia (Sunbiggin) }
9.	C. elenkinii	C. viguieri	Rivularia(Slapestone) }	Calothrix sp. D 258
10.	C. scopulorum	C. thermalis	Gloeotrichia(Brasside)	Rivularia (Slapestone)
11.	Calothrix sp. D 267	Calothrix sp. D 267	Rivularia (Sunbiggin)	C. thermalis
12.	Calothrix sp. D 258	Calothrix sp. D 258	C. brevissima	Rivularia (Sunbiggin)
13.	C. brevissima	C. gracilis	Calothrix sp. D 258	C. brevissima
14.	C. gracilis	C. brevissima	C. gracilis	C. gracilis
15.	Calothrix sp. D 255	Calothrix sp. D 255	Calothrix sp. D 255	Calothrix sp. D 255

The majority of cultures examined showed considerable variation in the values obtained for T_3, T_4 and in trichome length (Fig. 3.4). It seems likely that this variation may be due to the fact that trichome length (which is always numerically greater than trichome width) is incorporated into the expression:

$$T_4 = \frac{\frac{B-A}{B} \times 100}{L} .$$

The influence of length on T_4 can be examined by plotting curves of constant $\frac{B-A}{B} \times 100$. These theoretical curves were obtained by substituting theoretical, constant values of $\frac{B-A}{B}$ in the expression for T_4 e.g.

If $\frac{B-A}{B}$ is given a value of 30

then at trichome length of 100 μm $T_4 = \frac{30 \times 100}{100} = 30$

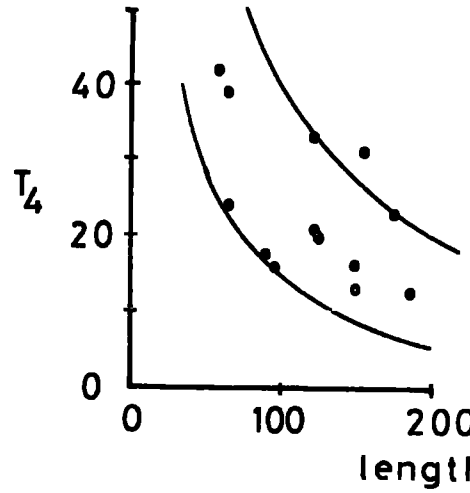
at a trichome length of 200 μm $T_4 = \frac{30 \times 100}{200} = 15$

at a trichome length of 300 μm $T_4 = \frac{30 \times 100}{300} = 10$

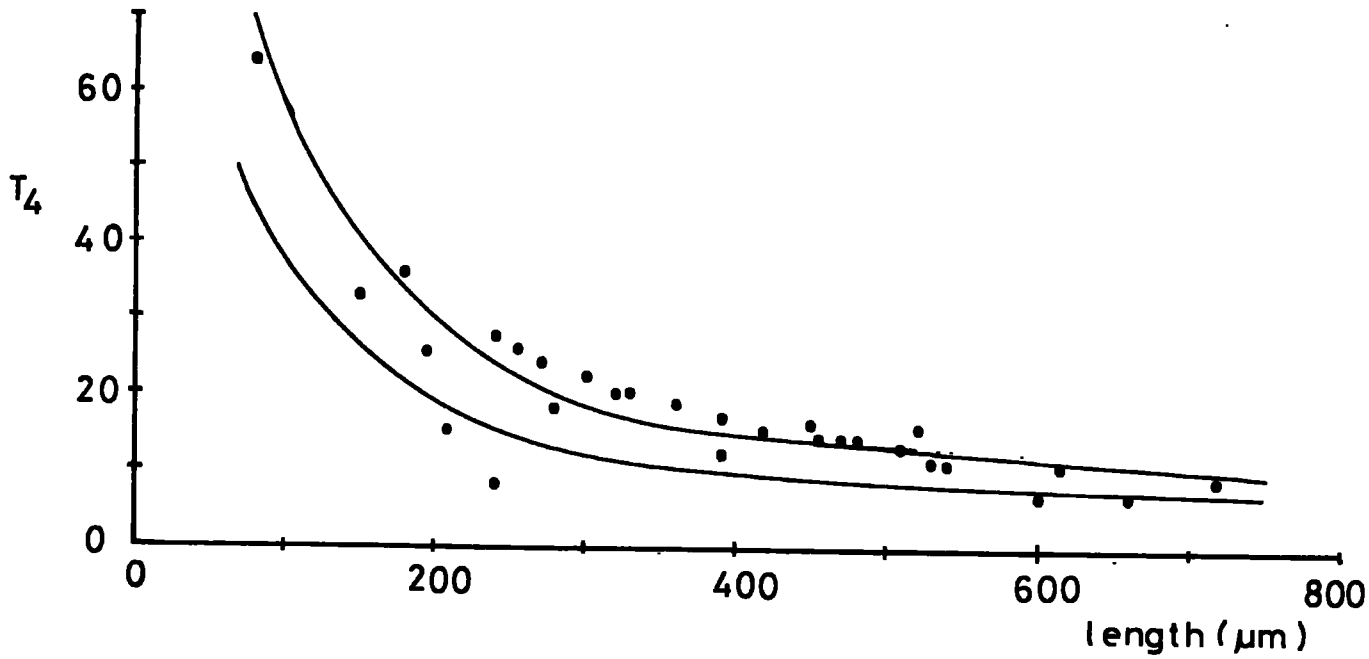
These theoretical curves were superimposed on the data in Fig. 3.4. As can be seen from Fig. 3.4 the data obtained showed similar trends to the theoretical curves and it seems likely that trichome length has considerable influence on the value obtained for the tapering index T_4. Great care will be needed when subculturing material to eliminate this and to obtain cultures in which the trichomes are of a similar age.

Fig. 3.4 Variation in trichome length and T_4 in 11 species of Calothrix and 4 Rivulariaceae. (Solid lines indicate theoretical curves of constant $\frac{B-A}{B}$ values.)

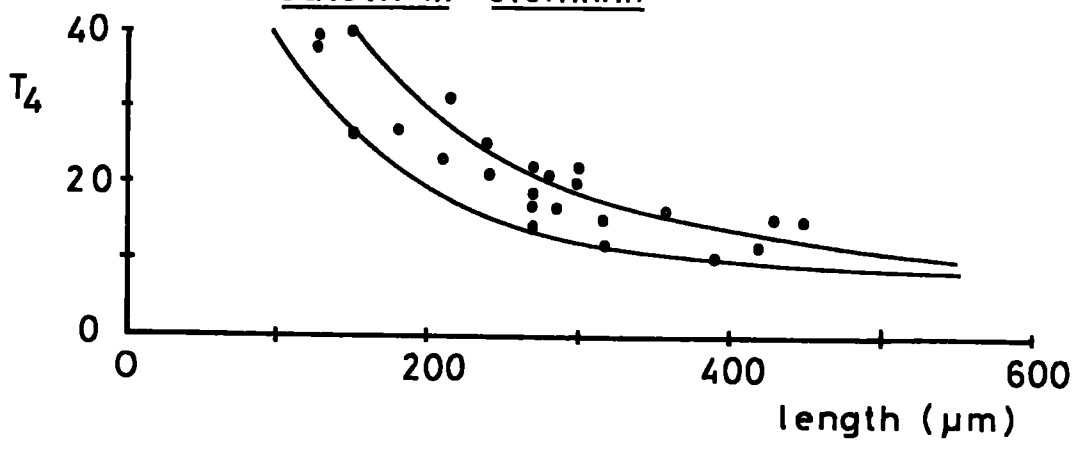
Calothrix brevissima



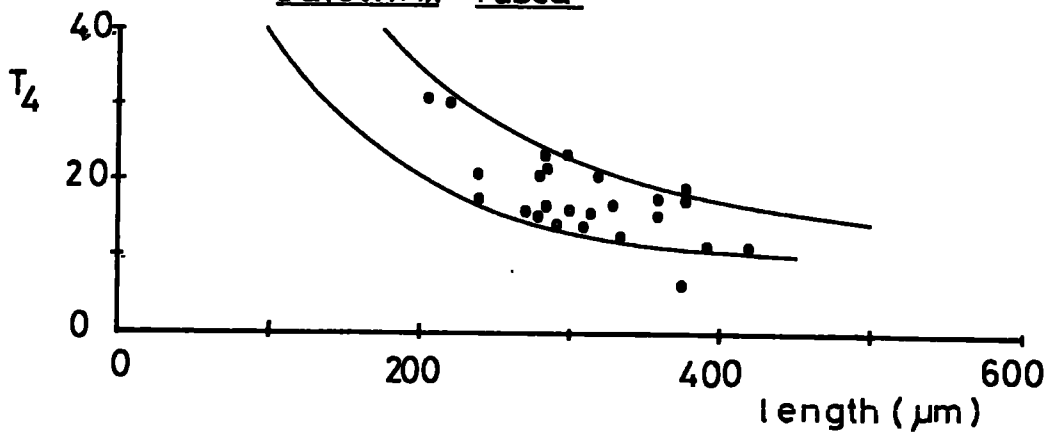
Calothrix desertica



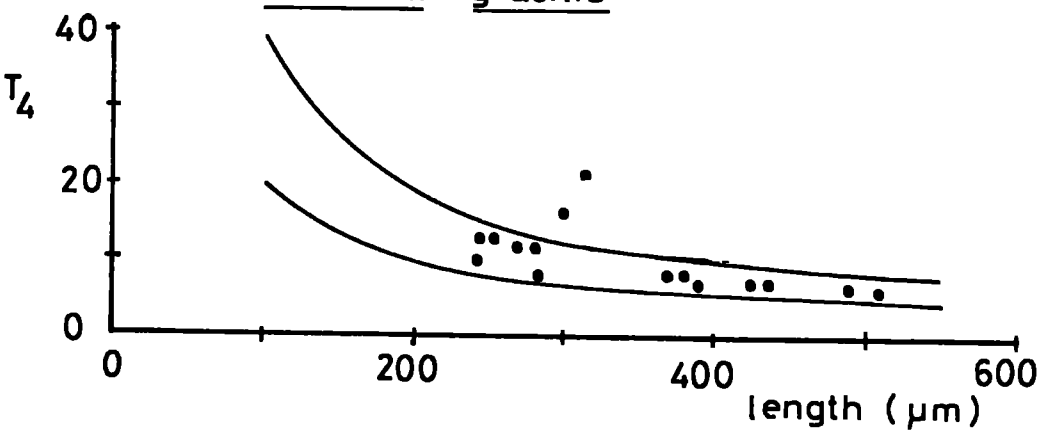
Calothrix elenkinii



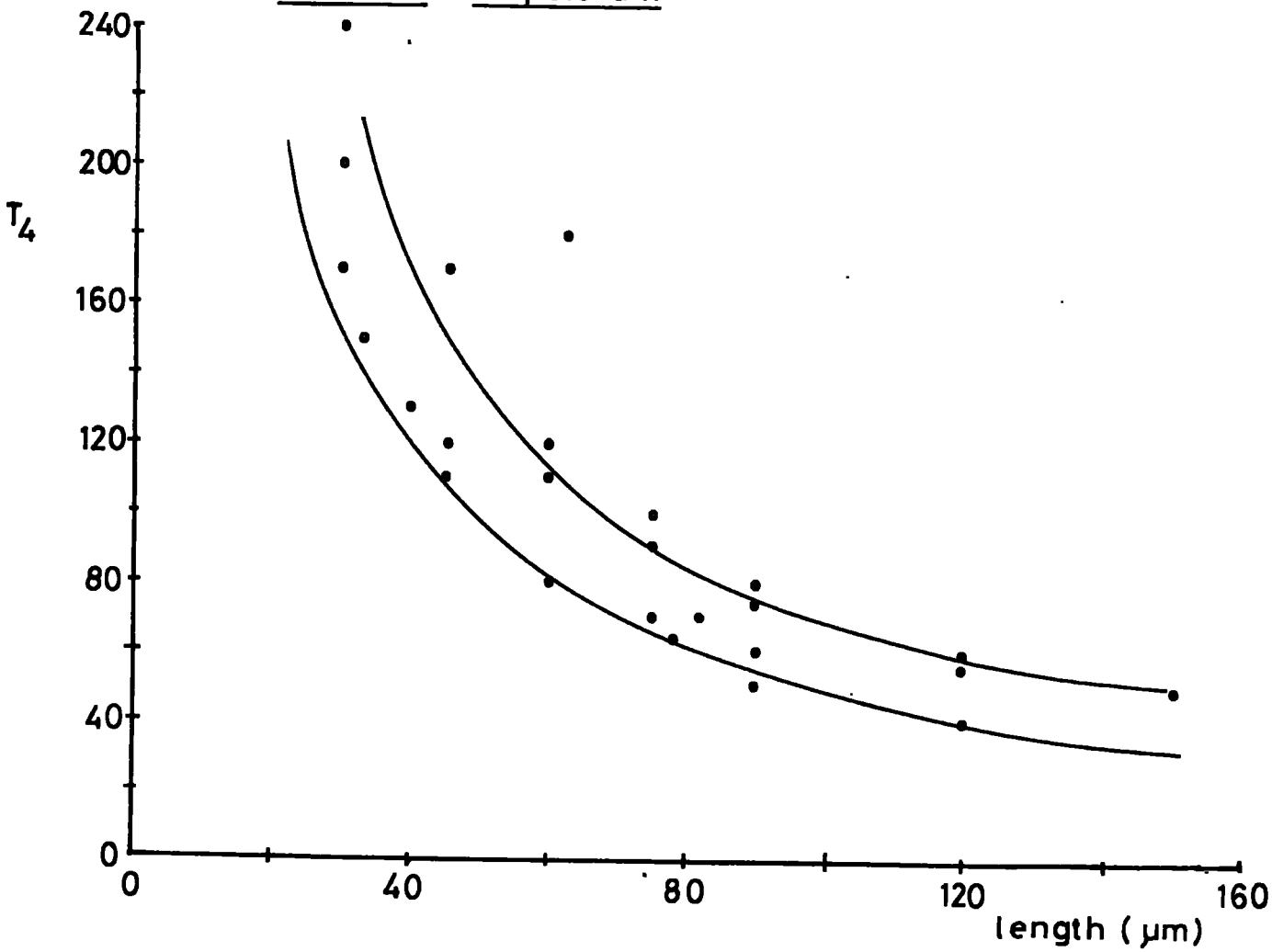
Calothrix fusca



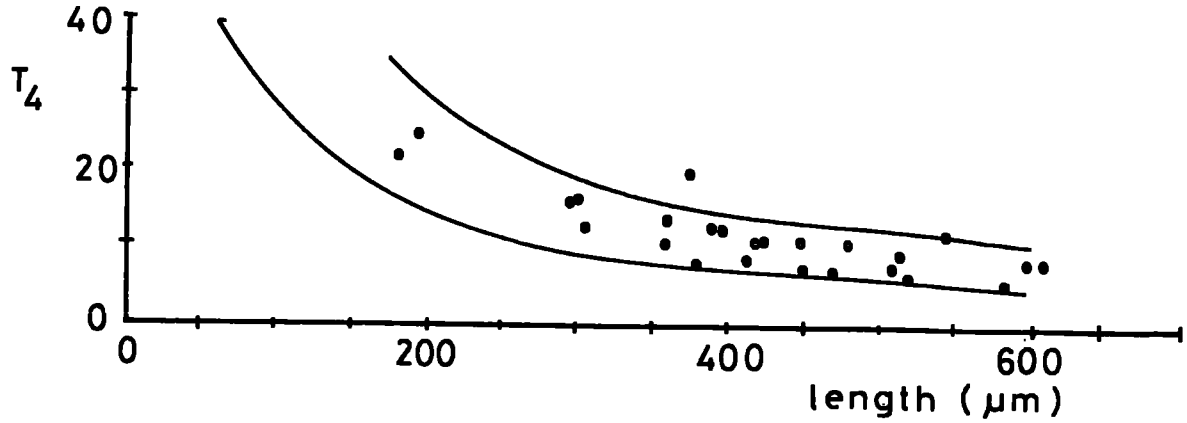
Calothrix gracilis



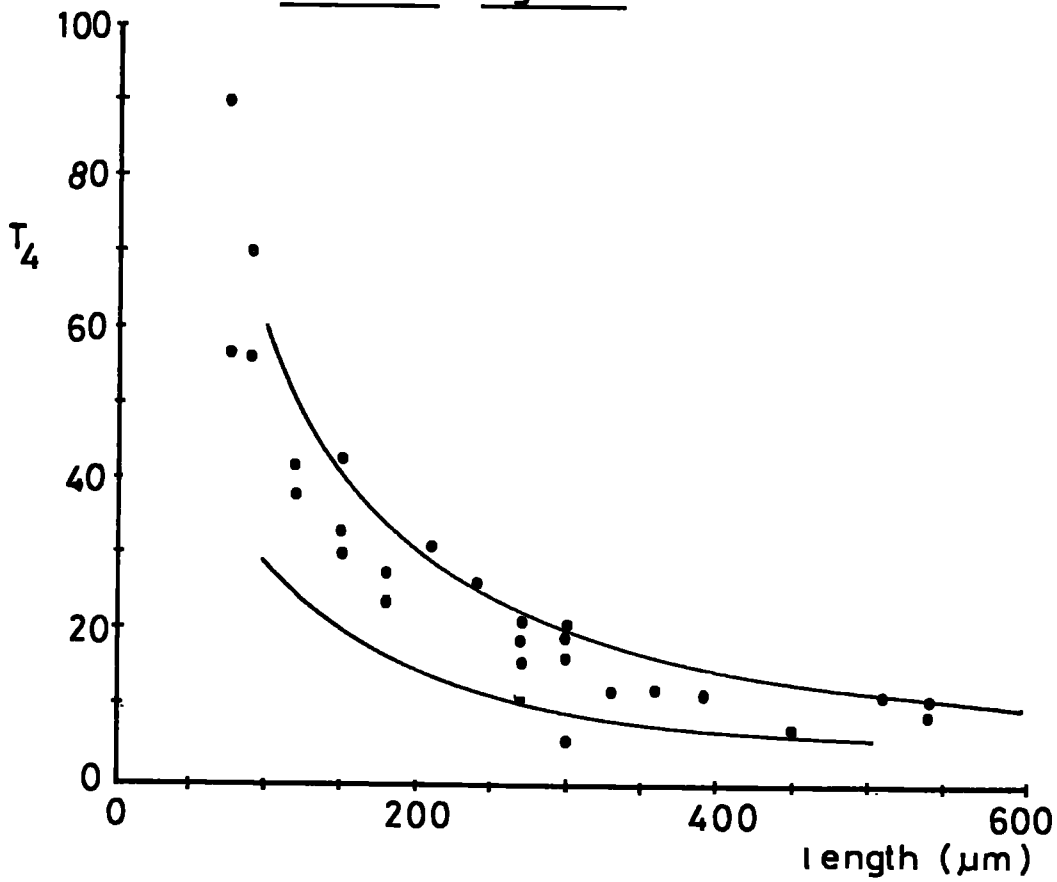
Calothrix scopulorum



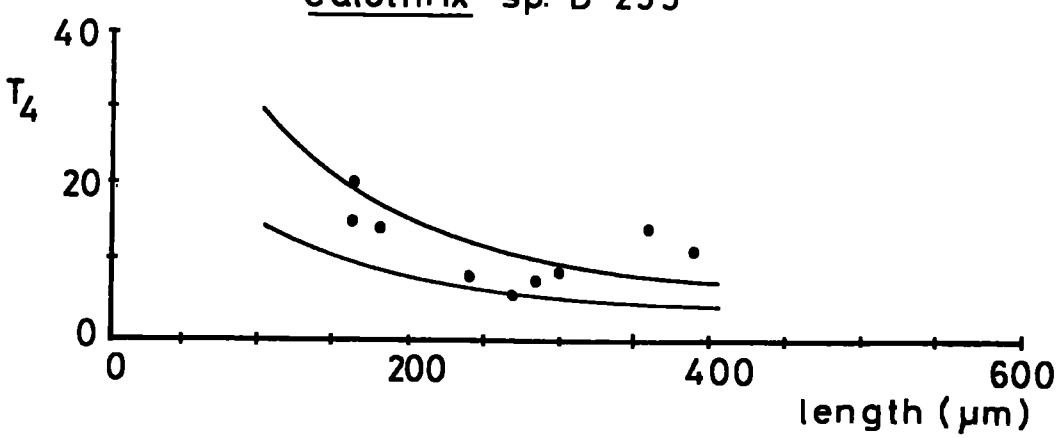
Calothrix thermalis



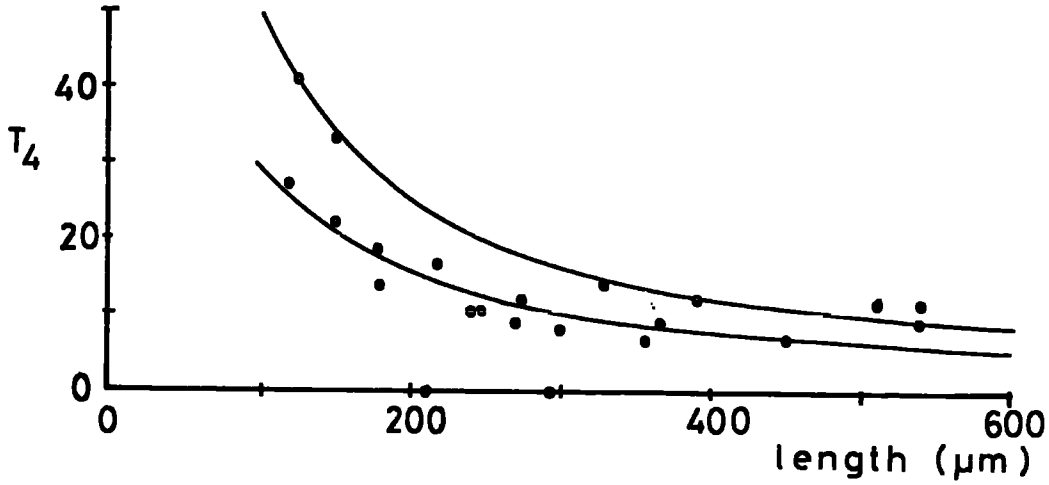
Calothrix viguieri



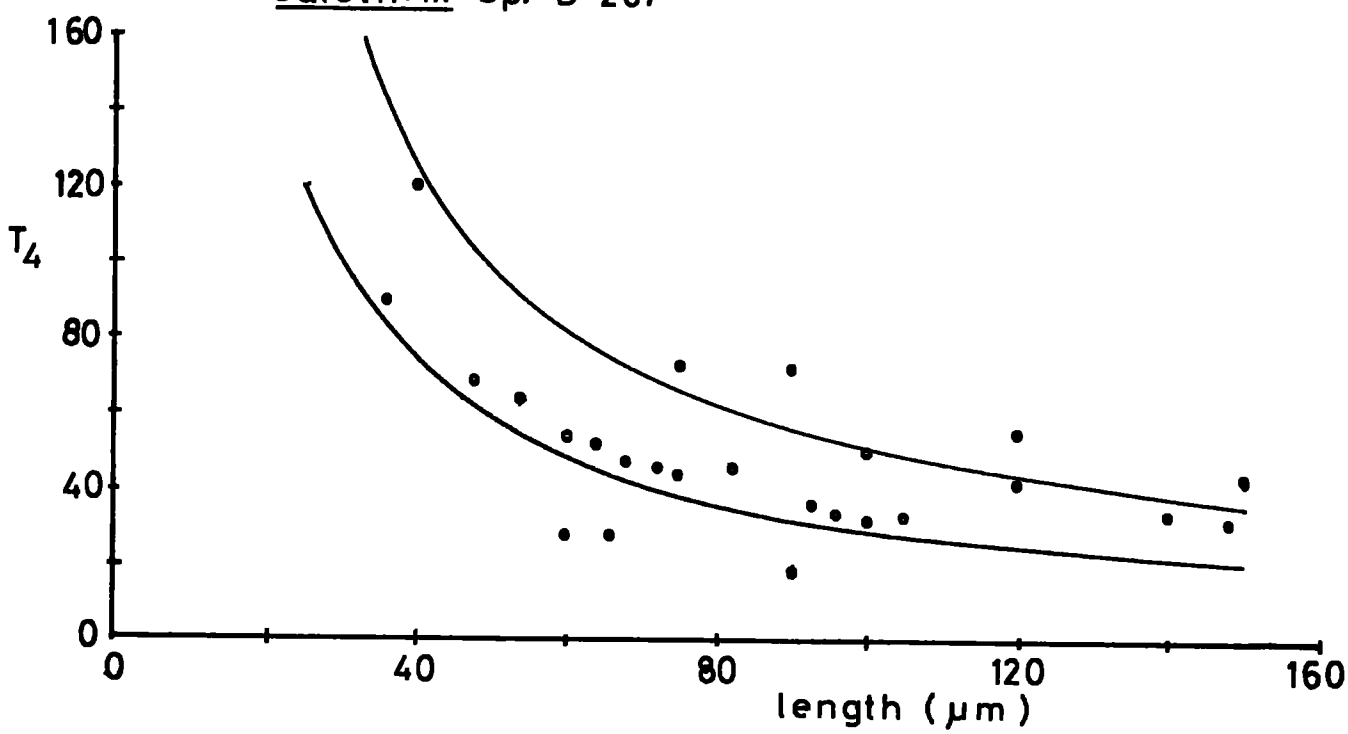
Calothrix sp. D 255



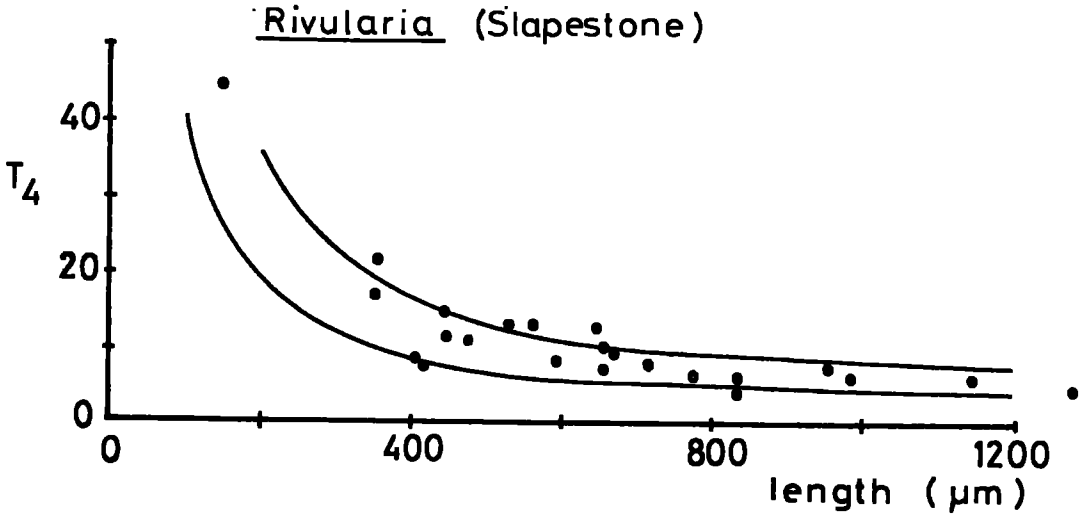
Calothrix sp. D 258



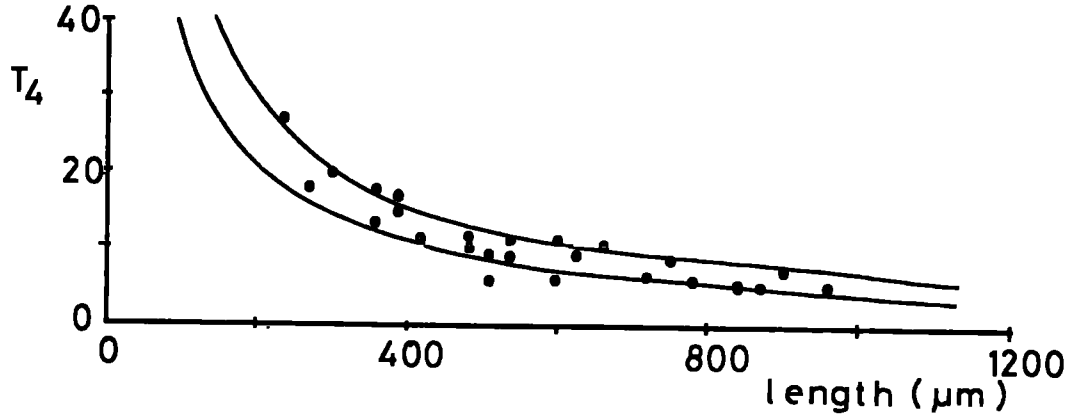
Calothrix sp. D 267



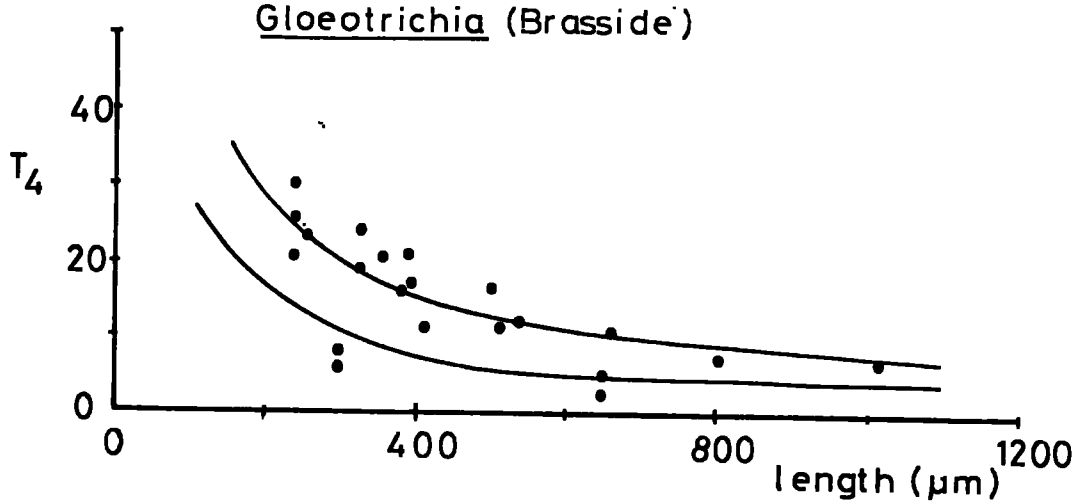
Rivularia (Slapestone)



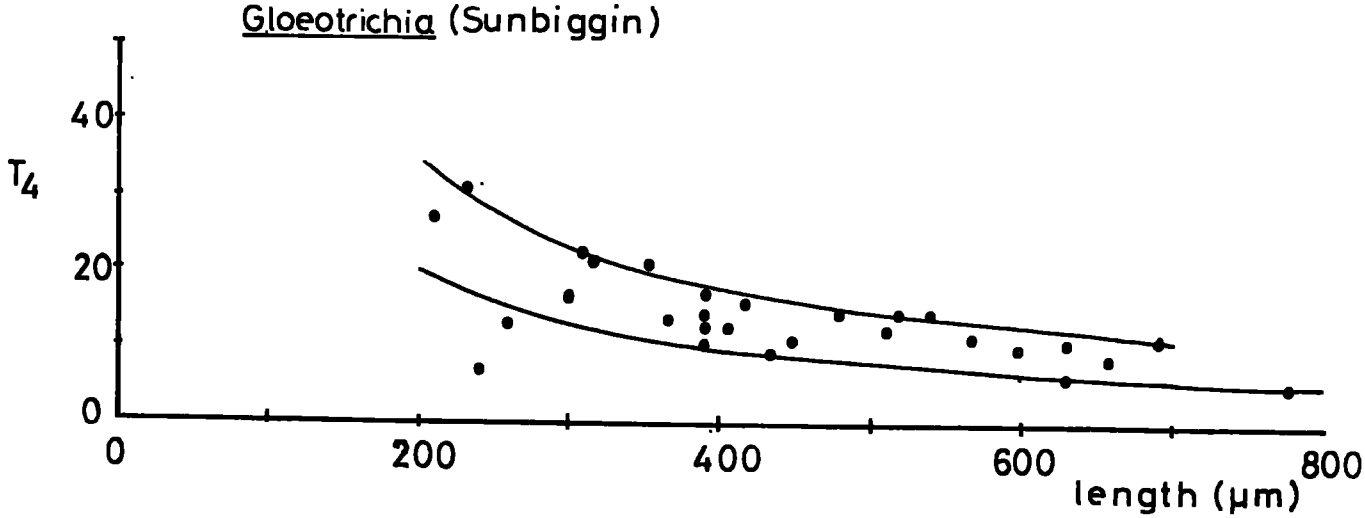
Rivularia (Sunbiggin)



Gloeotrichia (Brasside)



Gloeotrichia (Sunbiggin)



3.22 Detailed measurements of individual trichomes

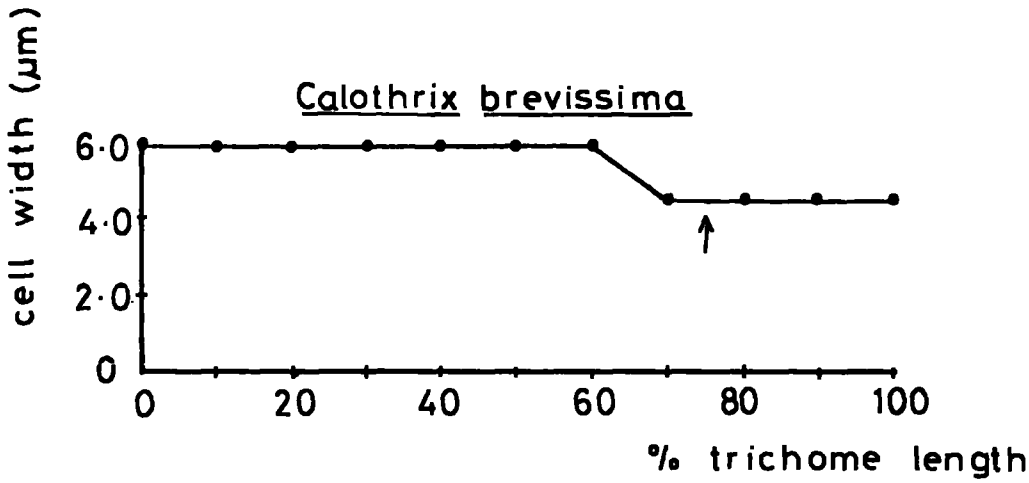
For simplicity and speed of measuring, it is useful to assume that tapering between the base and the apex is gradual i.e. the change in diameter occurs at a uniform rate. For a more detailed investigation of tapering it is necessary to record changes in the dimensions of individual cells in a single trichome. The cell dimensions of 10 species of Calothrix were measured. It seemed possible that this data could be summarised and/or made easier to collect by considering measurements at 10% intervals along the length of the trichomes. Information in this reduced form is shown graphically in Fig. 3.5. These figures give a rapid but approximate guide to the changes in shape of the trichomes. By indicating the total length of each trichome on the graph, all four indices of tapering can be calculated.

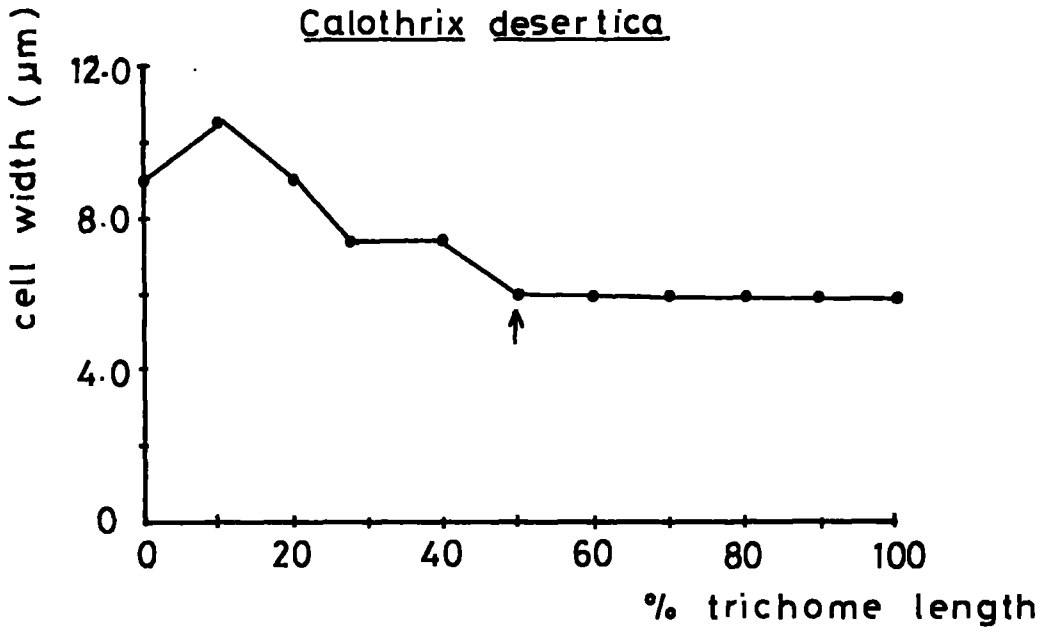
It may be of interest to indicate other features of the trichomes, such as where the hair begins to form. It may also be useful to indicate the position of the shortest cells in the trichome, as this is likely to include the meristematic zone. As described in Section 1.33, growth has been reported from a region at the base of the hair and near the base of the trichome. Short cells were observed in both regions (Fig. 3.5), the majority being beneath the narrowest region of the trichome.

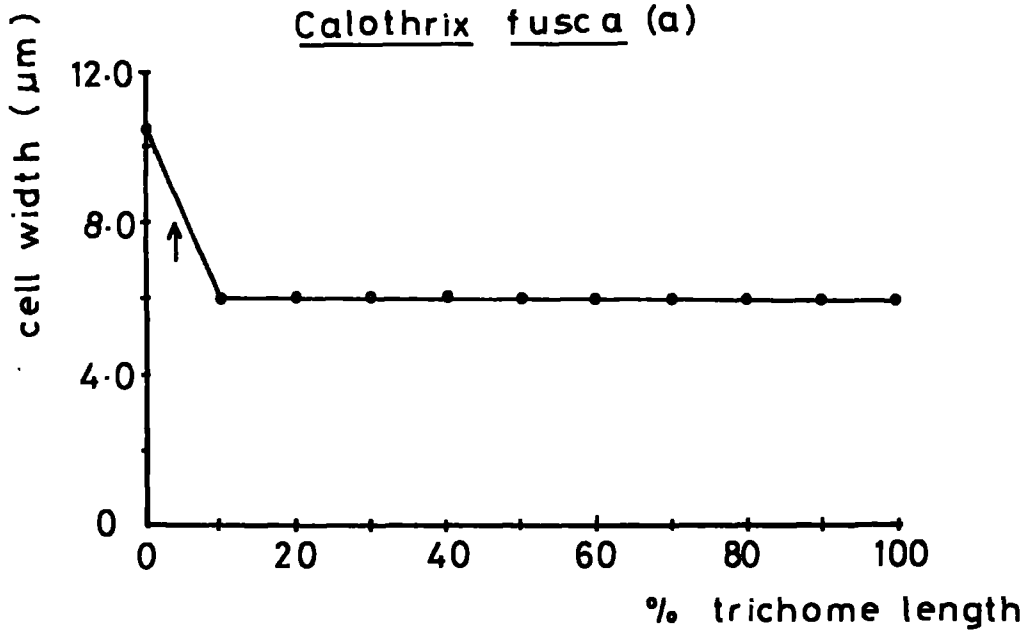
Although it is difficult to make generalisations based on such a small number of trichomes, the results serve to illustrate the complexity of any comparative work on tapering. In the majority of the cases illustrated, the change in the diameter of the trichome

Fig. 3.5 Cell widths at 10% intervals along individual trichomes. (Data given for 10 species of Calothrix.)

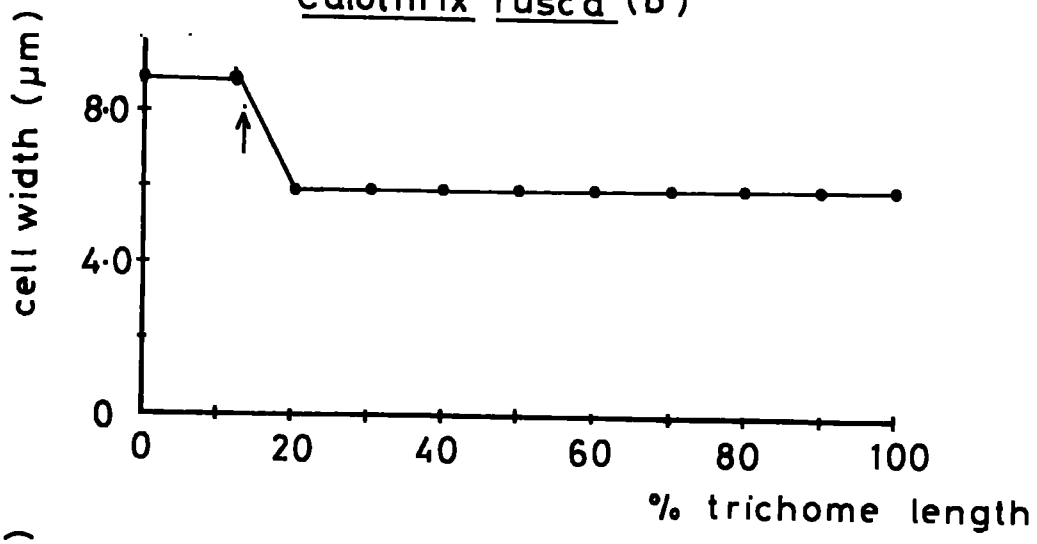
(shortest cells ↑)


Calothrix brevissima
cell width (µm)
6·0
4·0
2·0
0
0
20
40
60
80
100
% trichome length

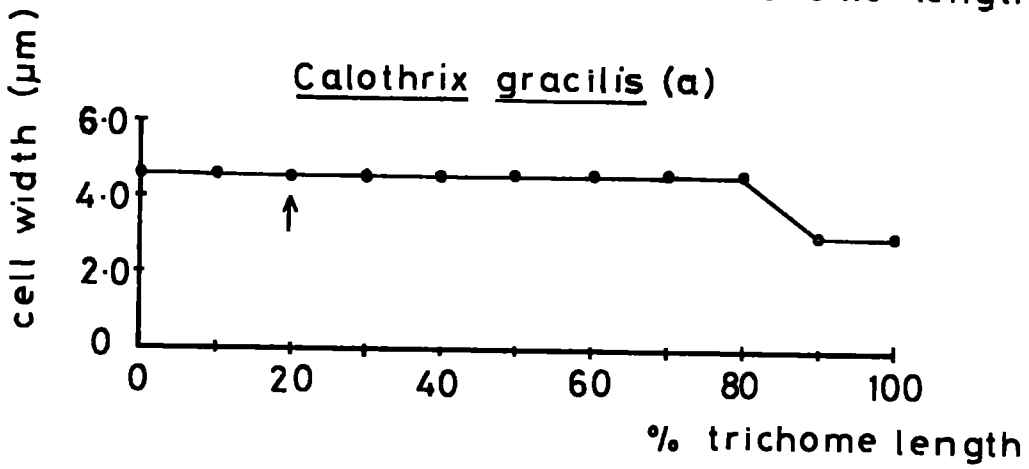

Calothrix desertica
cell width (µm)
12·0
8·0
4·0
0
0
20
40
60
80
100
% trichome length


Calothrix fusca (a)
cell width (µm)
12·0
8·0
4·0
0
0
20
40
60
80
100
% trichome length

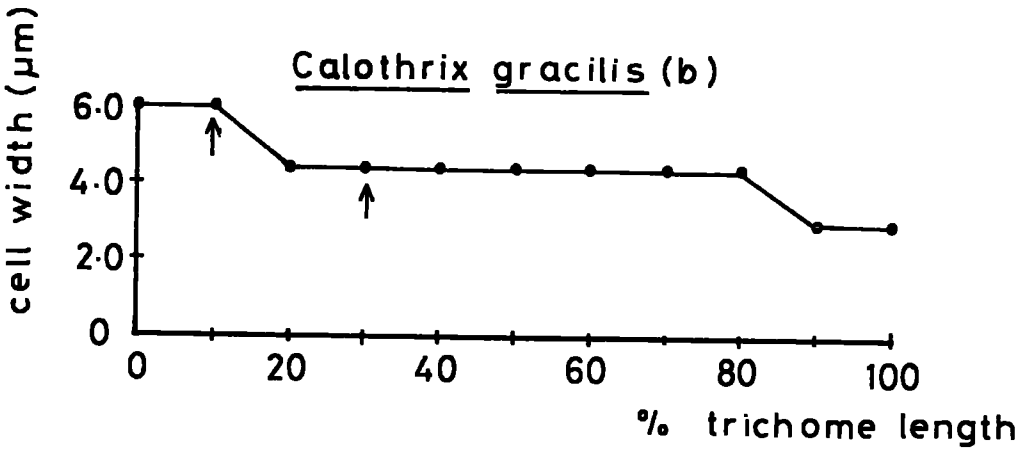
Calothrix fusca (b)



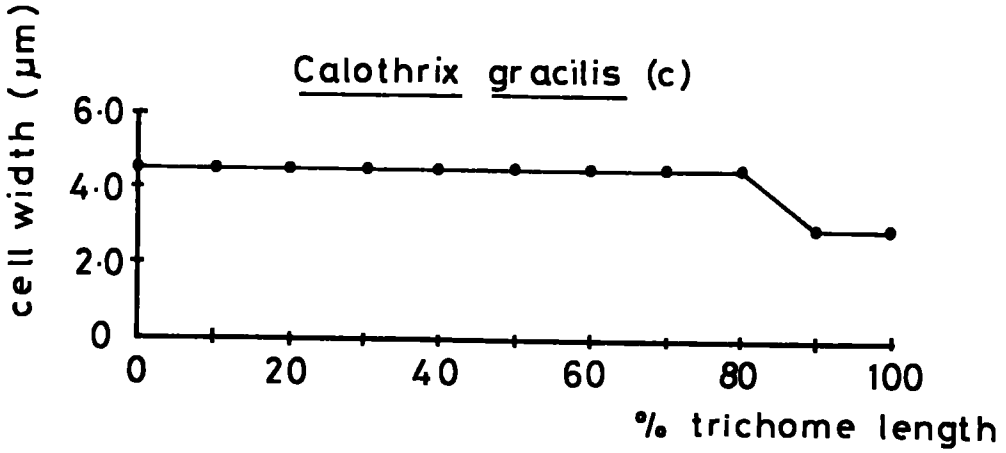
Calothrix gracilis (a)

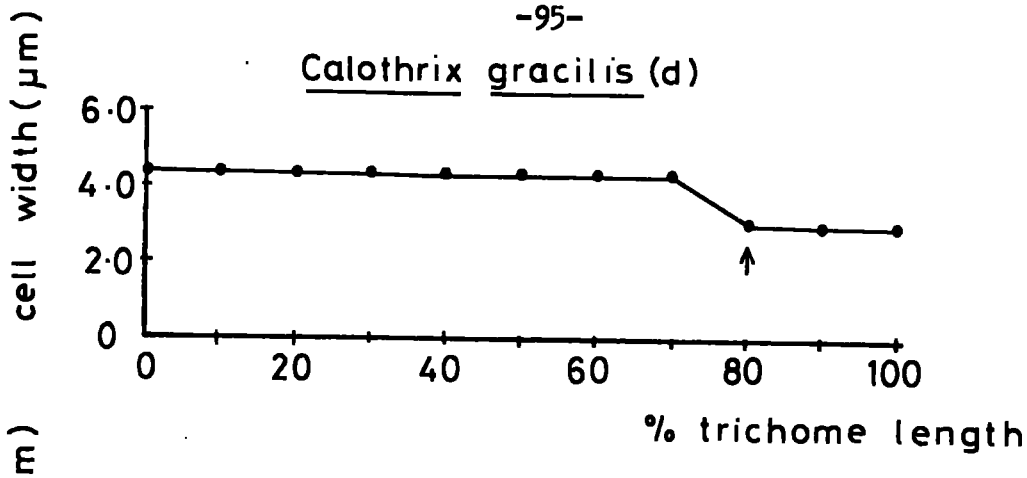


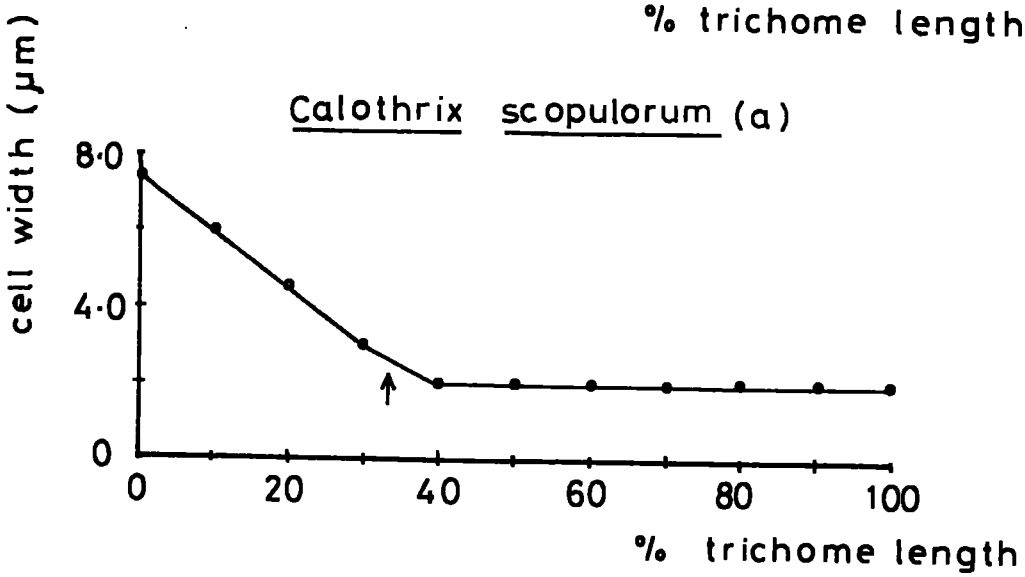
Calothrix gracilis (b)

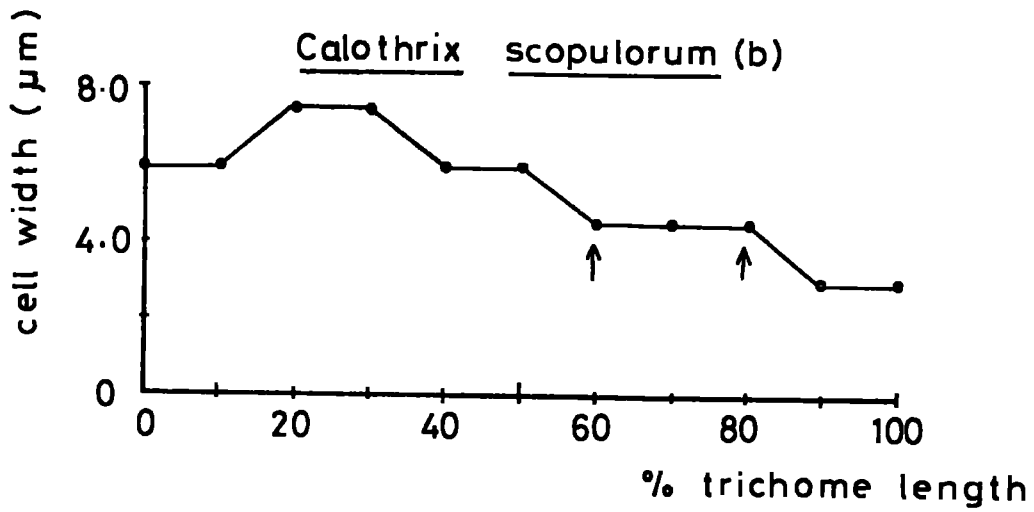


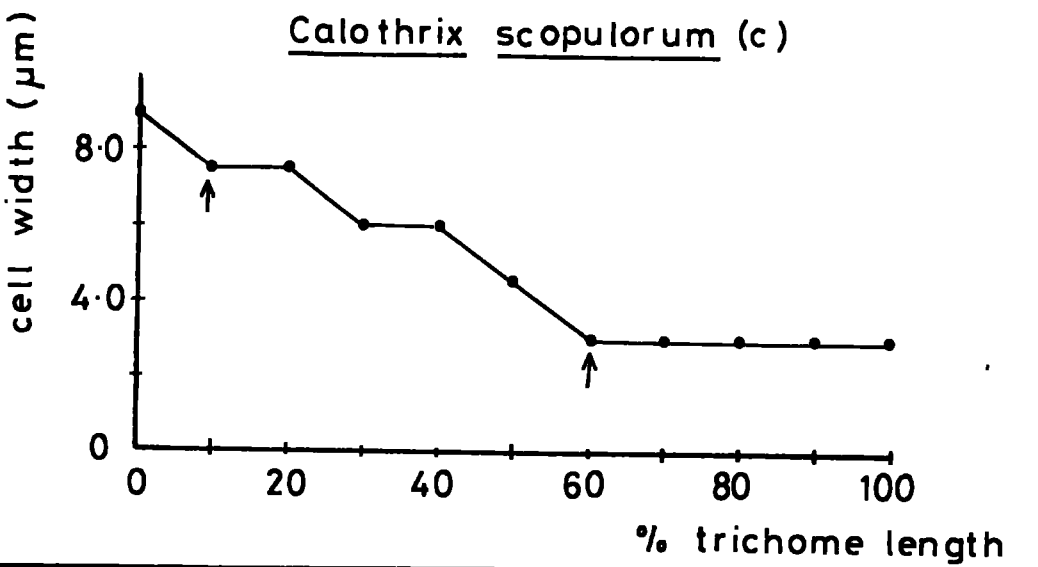
Calothrix gracilis (c)



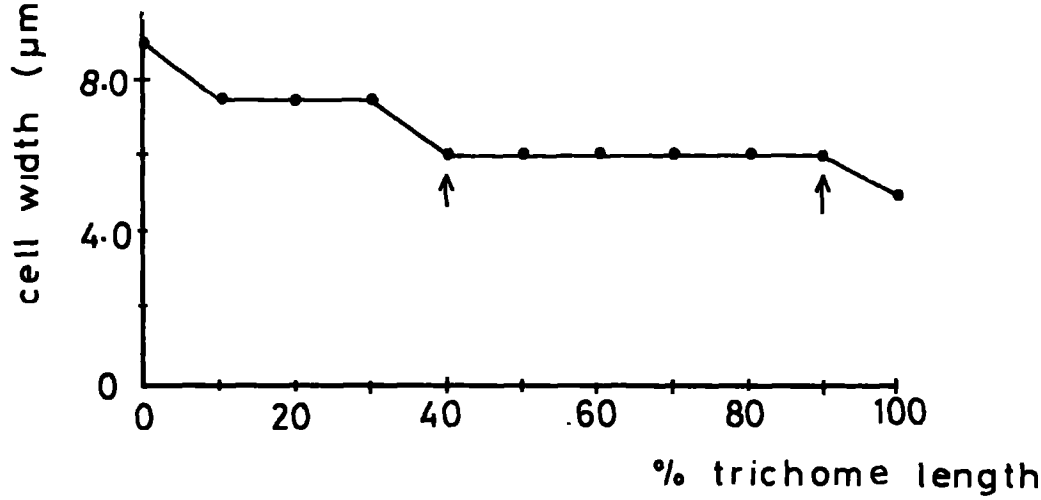

Calothrix gracilis (d)
cell width (µm)
6·0
4·0
2·0
0
0
20
40
60
80
100
% trichome length


Calothrix scopulorum (a)
cell width (µm)
8·0
4·0
0
0
20
40
60
80
100
% trichome length

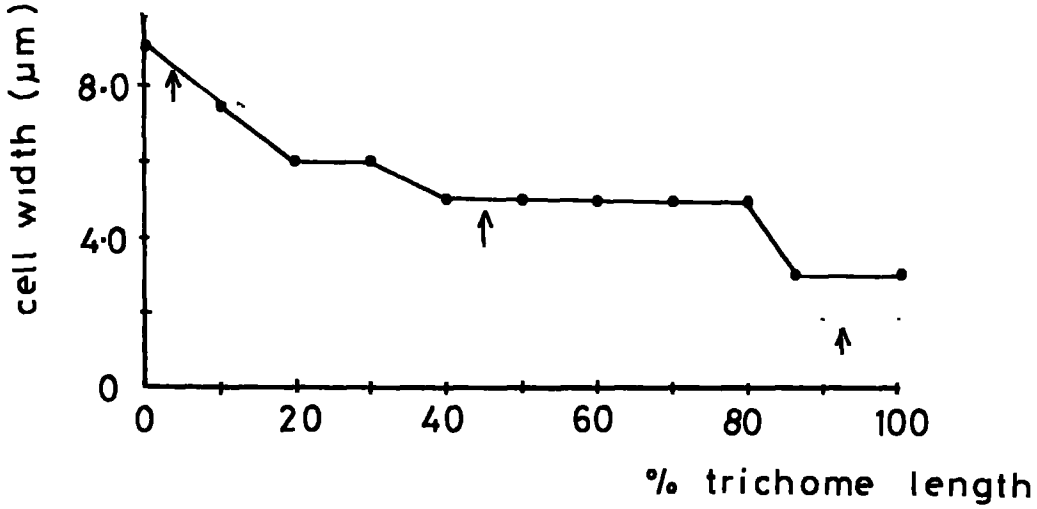

Calothrix scopulorum (b)
cell width (µm)
8·0
4·0
0
0
20
40
60
80
100
% trichome length


Calothrix scopulorum (c)
cell width (µm)
8·0
4·0
0
0
20
40
60
80
100
% trichome length

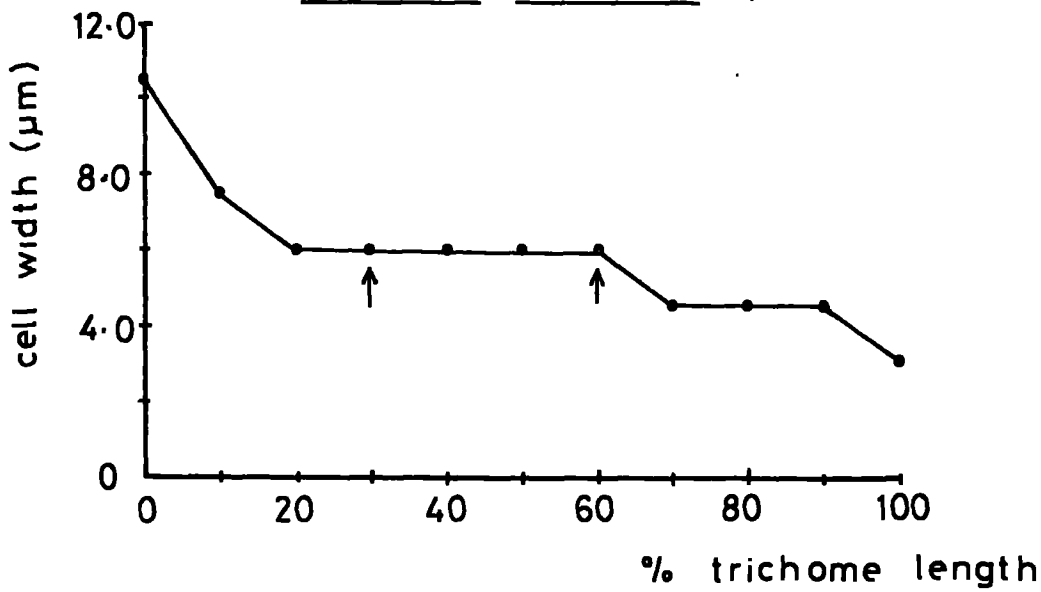
Calothrix thermalis (a)



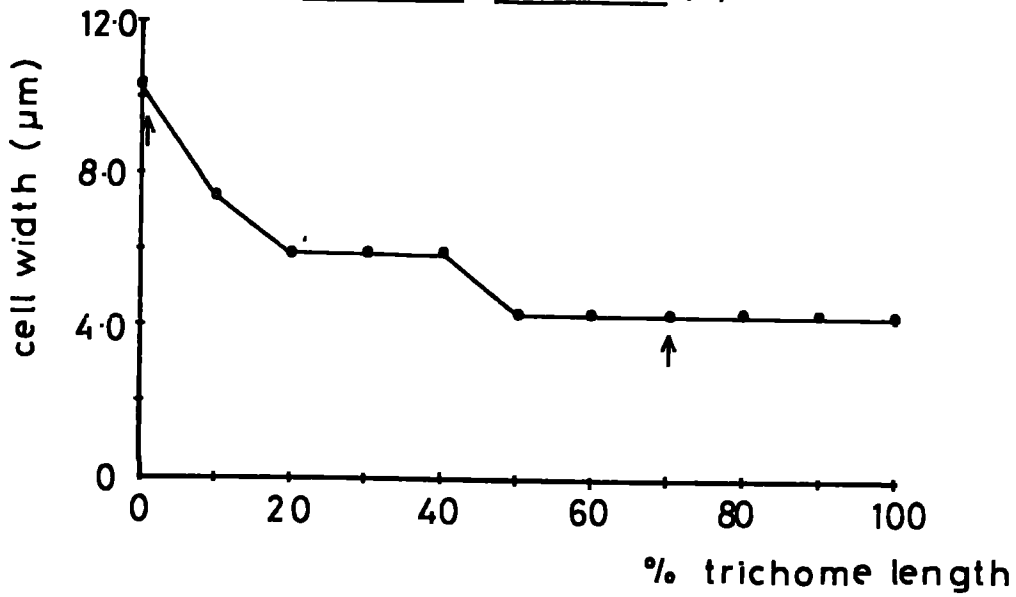
Calothrix thermalis (b)



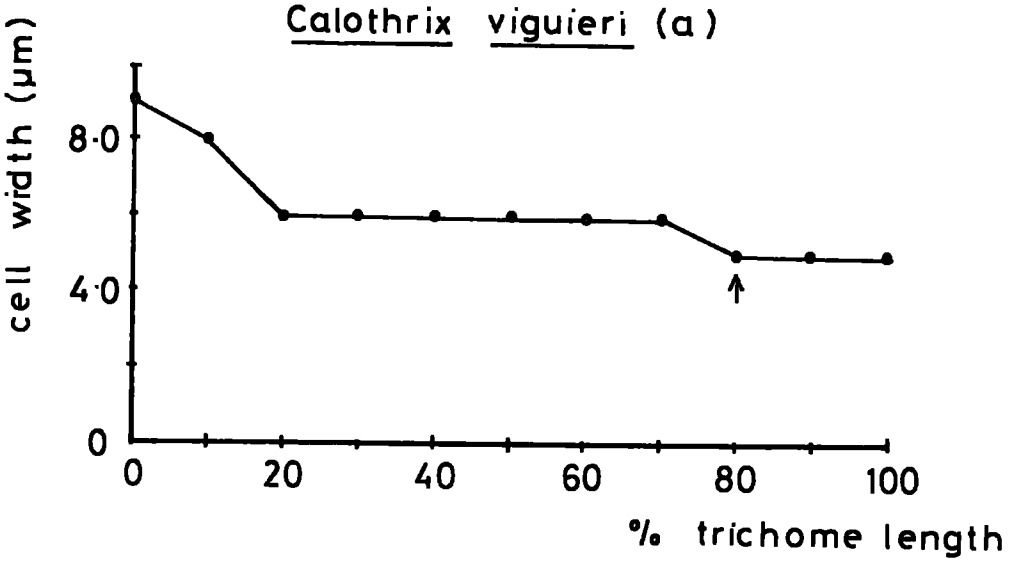
Calothrix thermalis (c)



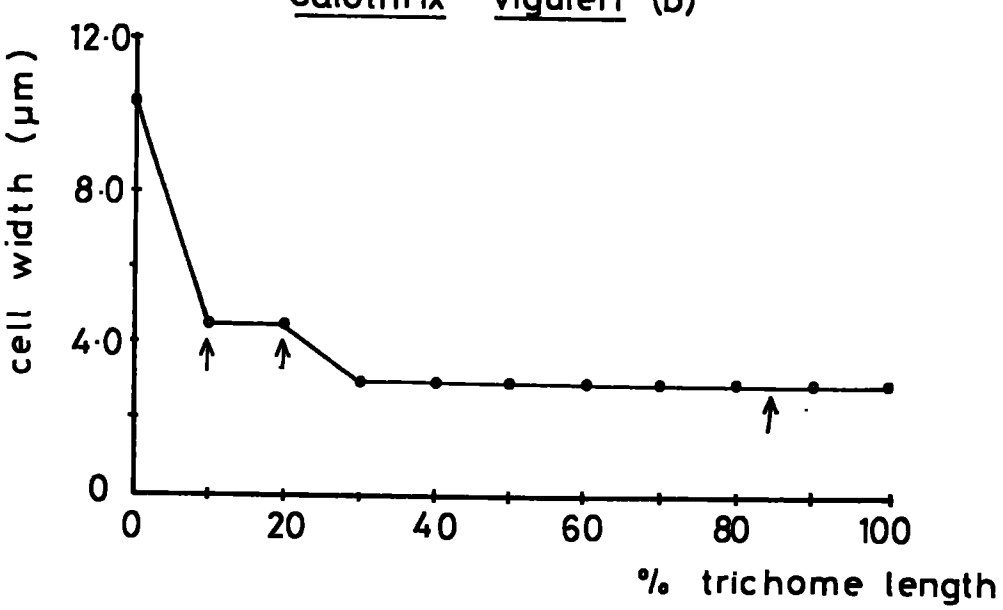
Calothrix thermalis (d)

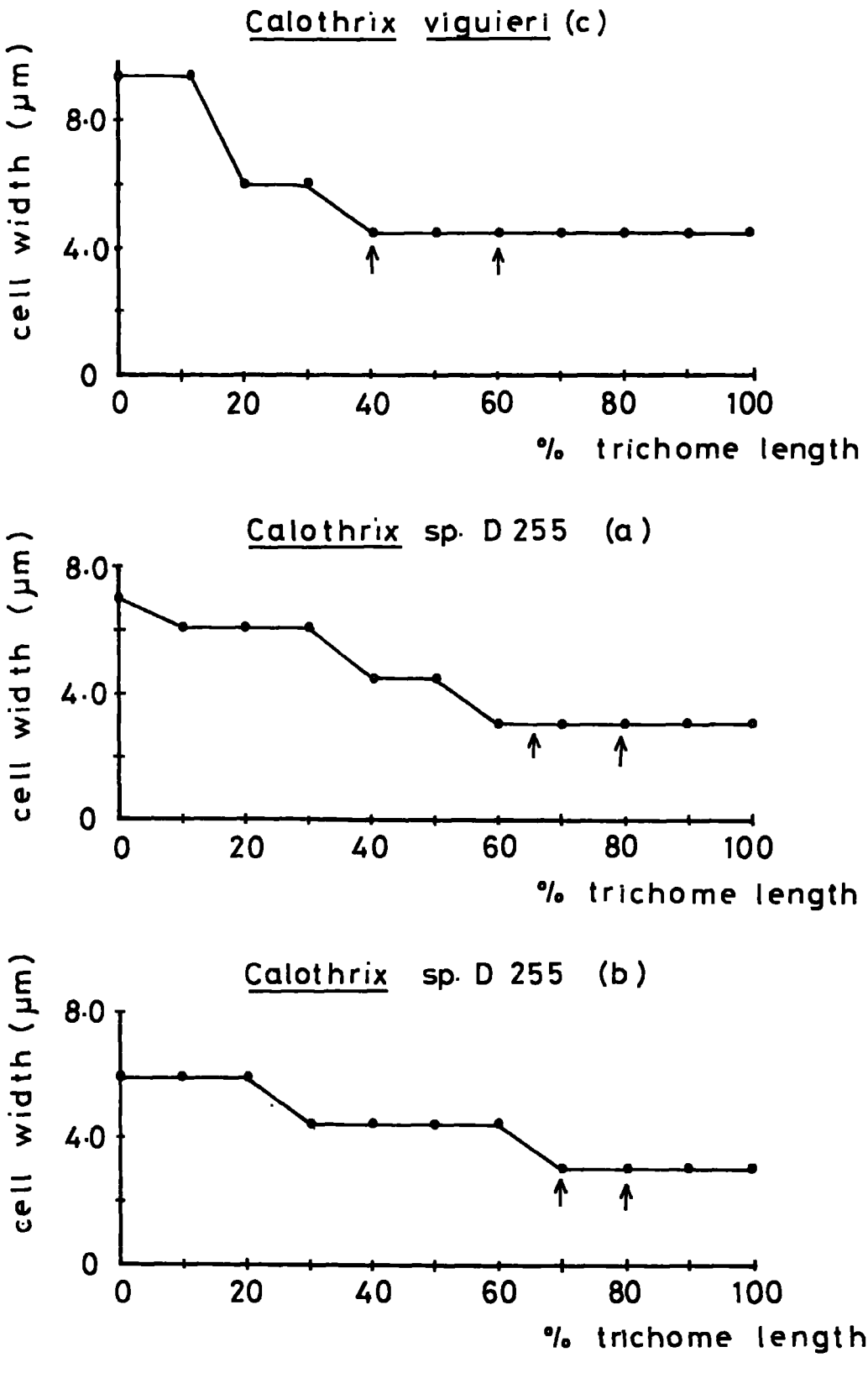


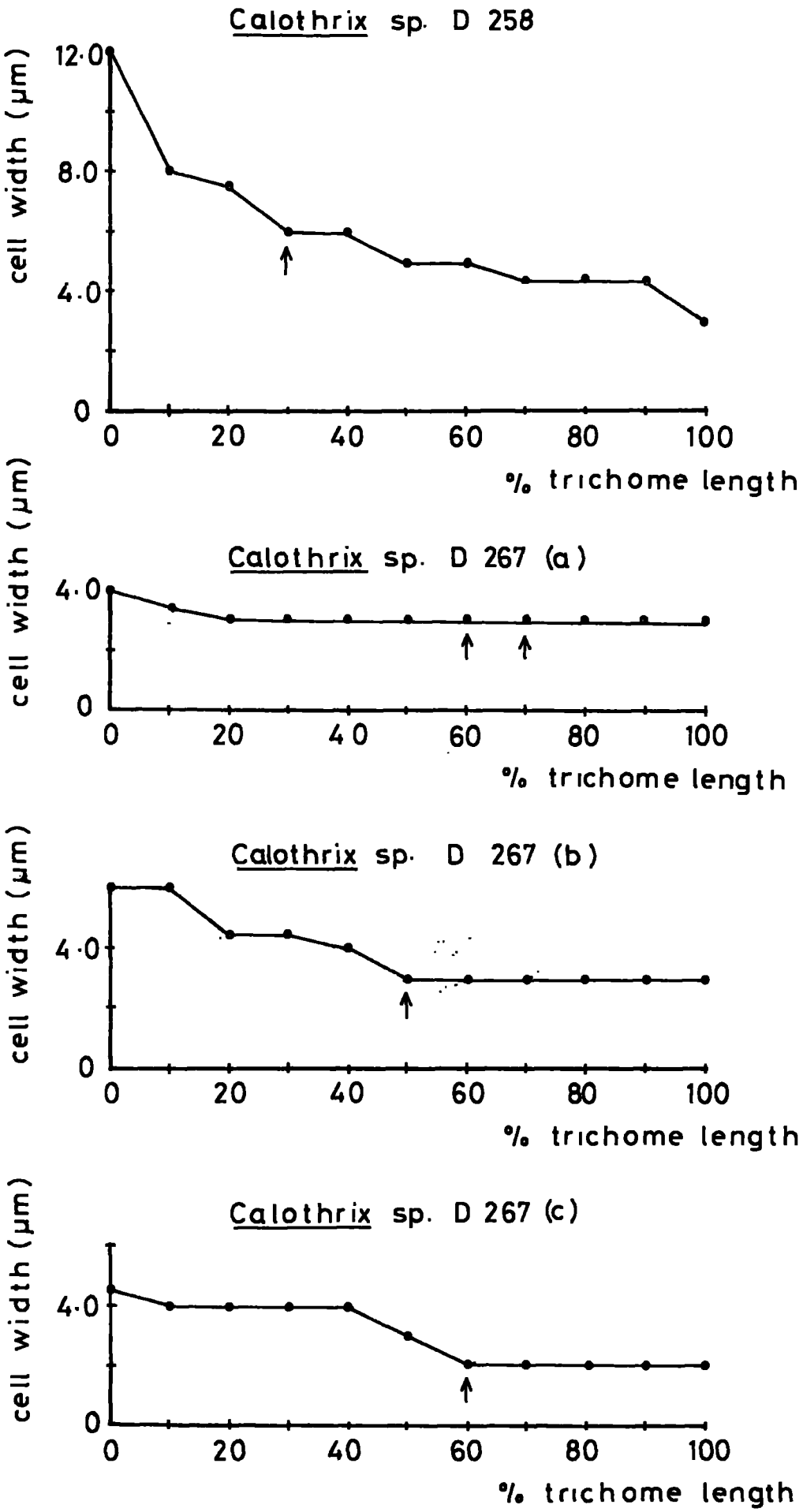
Calothrix viguieri (a)



Calothrix viguieri (b)




Calothrix viguieri (c)
cell width (µm)
8·0
4·0
0
0
20
40
60
80
100
% trichome length
Calothrix sp. D 255 (a)
cell width (µm)
8·0
4·0
0
0
20
40
60
80
100
% trichome length
Calothrix sp. D 255 (b)
cell width (µm)
8·0
4·0
0
0
20
40
60
80
100
% trichome length


Calothrix sp. D 258
cell width (µm)
12·0
8·0
4·0
0
0
20
40
60
80
100
% trichome length
Calothrix sp. D 267 (a)
cell width (µm)
4·0
0
0
20
40
60
80
100
% trichome length
Calothrix sp. D 267 (b)
cell width (µm)
4·0
0
0
20
40
60
80
100
% trichome length
Calothrix sp. D 267 (c)
cell width (µm)
4·0
0
0
20
40
60
80
100
% trichome length

appears to be more pronounced in the basal region. Tapering values for individual trichomes are given in Table 3.4 the trichomes being arranged in order of tapering in Table 3.5. Although very few trichomes were measured and the actual figures were variable, the results in Table 3.5 illustrate the difference arising from using total trichome length and using the length between the regions of maximum width and minimum width.

Having considered changes associated with cell width, changes in external surface area and volume of cells of individual trichomes, were studied. The mean external surface area and volume of the basal and apical cells of 9 species of Calothrix and 2 Gloeotrichia species are given in Table 3.6. Changes in the external surface area and volume of cells in single filaments of these species are shown in Fig. 3.6.

In most cases the actual surface area and volume of the basal cell were greater than those of the apical cell, the exception being the Gloeotrichia from Sunbiggin. In every case the ratio of surface area:volume was greater for the apical cell, as shown in Table 3.7 (see also Section 3.3).

3.3 The hair

The hair of members of the Rivulariaceae was described in Section 1.23 as a long series of narrow cells which are colourless or vacuolate. In the literature, the hair has been described qualitatively, in terms of being present or absent and long or short (Geitler 1932), but no quantitative measurements have been given.

Table 3.4 Tapering values (calculations based on Fig. 3.5)

species		T_1	T_2	T_3	T_4	T_5*	T_6*
Calothrix brevissima		1.5	25	2.33	38	23.3	385
C. desertica		3.0	43	5.6	53	9.2	103
C. fusca	(1)	4.5	43	1.7	17	17.5	166
	(2)	3.0	30	3.4	34	34.0	376
C. gracilis	(1)	1.5	30	2.6	51	26.0	575
	(2)	3.0	50	3.1	52	3.5	58
	(3)	1.5	30	5.9	130	60.0	1300
	(4)	1.5	30	0.3	6	3.0	68
C. scopulorum	(1)	5.5	70	5.5	70	13.8	183
	(2)	3.0	50	8.5	131	12.1	201
	(3)	6.0	70	11.4	134	18.9	210
C. thermalis	(1)	6.0	67	2.7	30	2.0	23
	(2)	6.0	67	8.4	94	9.5	105
	(3)	7.5	75	4.3	41	4.1	39
	(4)	6.0	60	3.4	34	7.2	68
C. viguieri	(1)	4.5	50	1.2	13	1.6	18
	(2)	7.5	71	2.2	21	7.4	70
	(3)	4.5	50	1.9	22	6.5	73
Calothrix sp. D255	(1)	4.0	60	4.1	62	6.8	98
	(2)	3.0	50	1.8	30	3.6	61
Calothrix sp. D258		9.0	75	4.6	38	46.0	39
Calothrix sp. D267	(1)	1.0	25	0.9	22	3.7	92
	(2)	3.0	50	3.7	60	7.9	153
	(3)	2.5	56	4.9	11	8.2	193

* $T_5 = T_3$ and $T_6 = T_4$ but calculated with L = length between region of trichome with maximum and minimum width.

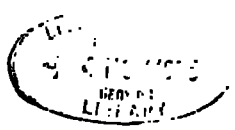


Table 3.5 Tapering order (based on mean values from Table 3.4)

	T_1	T_2	T_3	T_4	T_5	T_6
1.	*Calothrix thermalis*	*C. thermalis*	*C. scopulorum*	*C. scopulorum*	*Calothrix* sp.D258	*C. gracilis*
2.	*Calothrix* sp. D258	*C. scopulorum*	*C. desertica*	*C. gracilis*	*C. fusca*	*C. brevissima*
3.	*C. scopulorum*	*Calothrix* sp.D255	*C. thermalis*	*C. desertica*	*C. gracilis*	*C. fusca*
4.	*C. viguieri*	*C. viguieri*	*Calothrix* sp.D258	*C. thermalis*	*C. scopulorum*	*C. scopulorum*
5.	*C. desertica*	*Calothrix* sp.D258	*Calothrix* sp.D255	*Calothrix* spD267	*C. brevissima*	*Calothrix* sp.D267
6.	*C. fusca*	*C. desertica*	*C. gracilis*	*Calothrix* spD255	*C. viguieri*	*C. viguieri*
7.	*Calothrix* sp. D255	*C. fusca*	*C. fusca*	*C. brevissima*	*Calothrix* sp.D267	*Calothrix* sp.D255
8.	*C. gracilis*	*C. gracilis*	*Calothrix* sp.D267	*Calothrix* spD258	*C. thermalis*	*C. thermalis*
9.	*Calothrix* sp. D267	*Calothrix* sp.D267	*C. viguieri*	*C. fusca*	*Calothrix* sp.D255	*Calothrix* sp.D258
10.	*C. brevissima*	*C. brevissima*	*C. brevissima*	*C. viguieri*	*C. desertica*	*C. desertica*

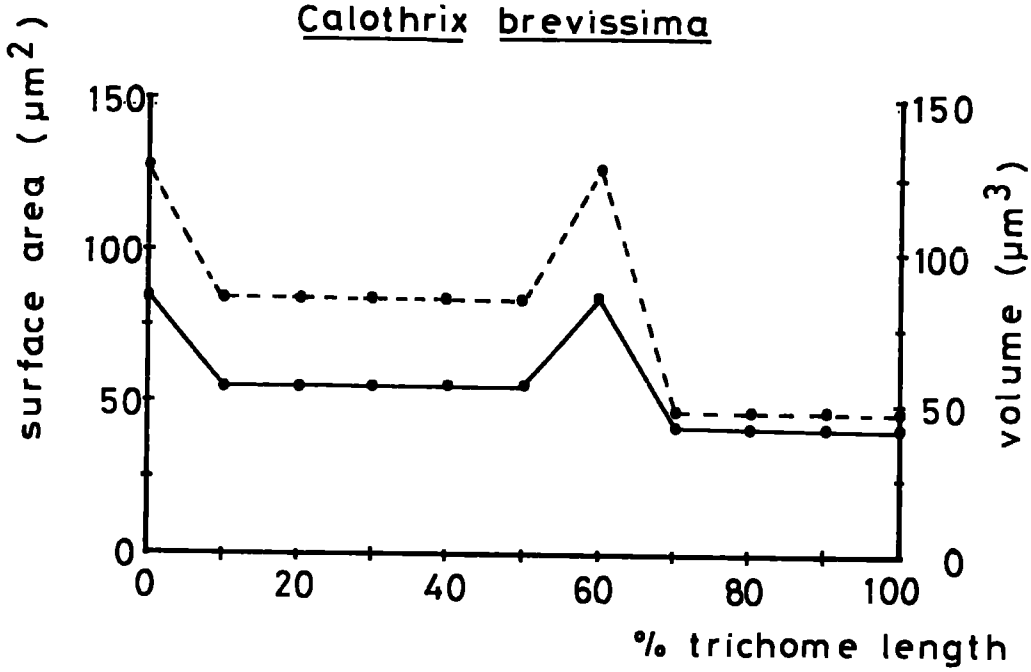
Table 3.6 External surface area and volume measurements
(means and standard errors)

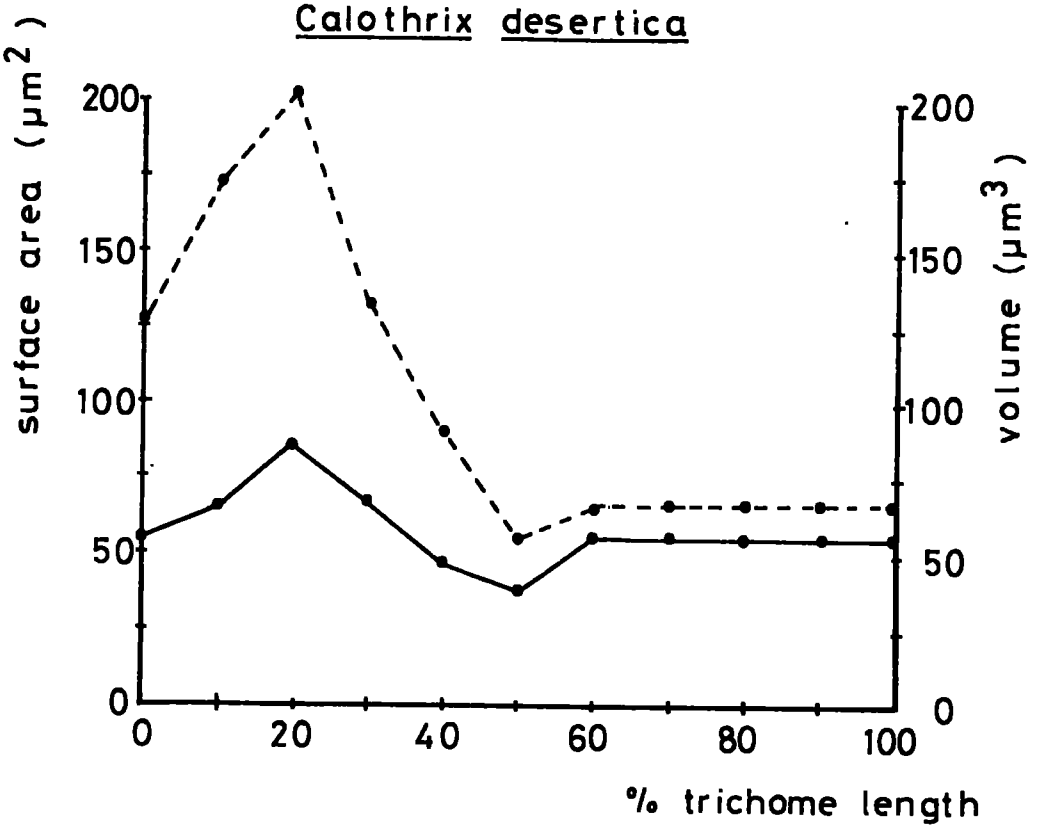
species	basal cell		apical cell	
	surface area (μm^2)	volume (μm^3)	surface area (μm^2)	volume (μm^3)
Calothrix brevissima	98.03 ± 5.42	162.67 ± 14.90	65.82 ± 4.26	94.89 ± 8.45
C. fusca	140.21 ± 5.68	360.23 ± 19.25	89.23 ± 4.38	114.55 ± 9.51
C. gracilis	105.34 ± 2.69	123.73 ± 5.63	40.30 ± 2.73	40.36 ± 3.66
C. scopulorum	63.78 ± 2.84	100.79 ± 6.42	18.75 ± 1.91	11.49 ± 1.48
C. thermalis	169.67 ± 6.18	431.85 ± 24.70	48.70 ± 2.69	63.29 ± 5.25
C. viguieri	240.83 ± 6.89	669.76 ± 32.10	116.73 ± 4.63	159.41 ±10.30
Calothrix sp.D255	122.59 ± 3.45	192.51 ± 8.71	89.08 ± 3.38	127.17 ± 6.61
Calothrix sp.D258	55.85 ± 2.57	75.86 ± 5.00	45.95 ± 1.94	43.71 ± 3.16
Calothrix sp.D267	79.18 ± 5.30	89.90 ± 7.78	23.75 ± 1.30	16.25 ± 1.12
Gloeotrichia(Brasside)	195.22 ± 1.15	366.20 ± 3.69	101.89 ± 1.50	95.68 ± 7.39
Gloeotrichia(Sunbiggin)	232.11 ± 5.86	595.72 ± 26.10	256.23 ± 3.66	292.96 ± 7.35

Fig. 3.6 Changes in the external surface area and volume of cells in individual trichomes. (Data given for 10 species of Calothrix.)

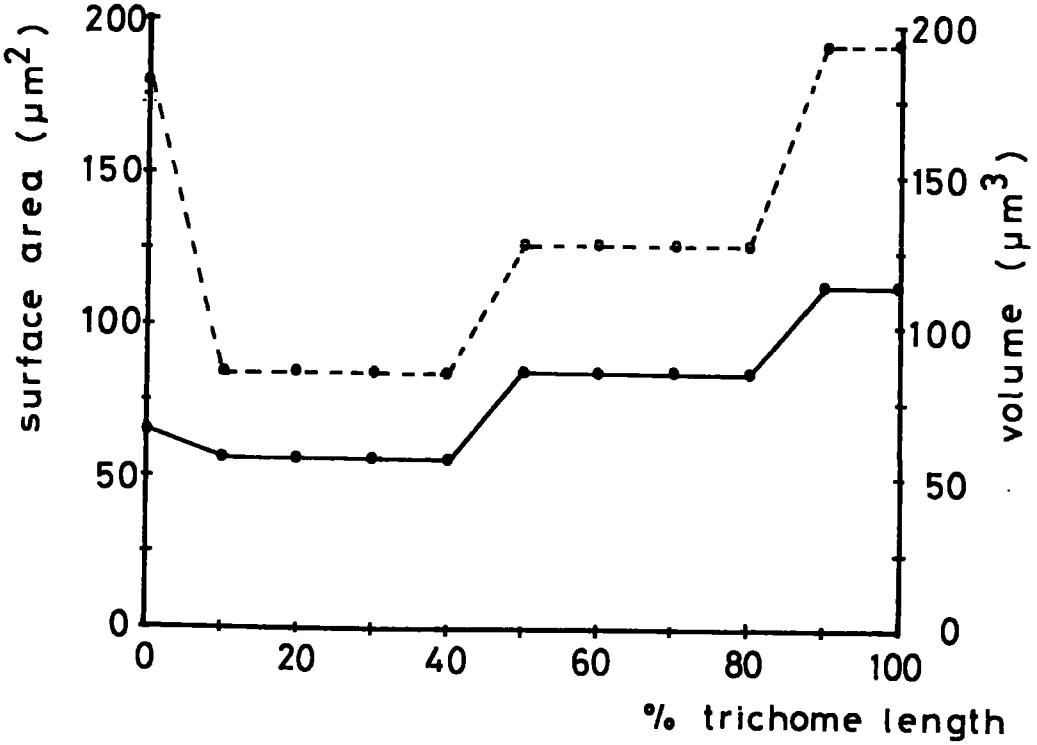
———— external surface area

- - - - - - - - - volume

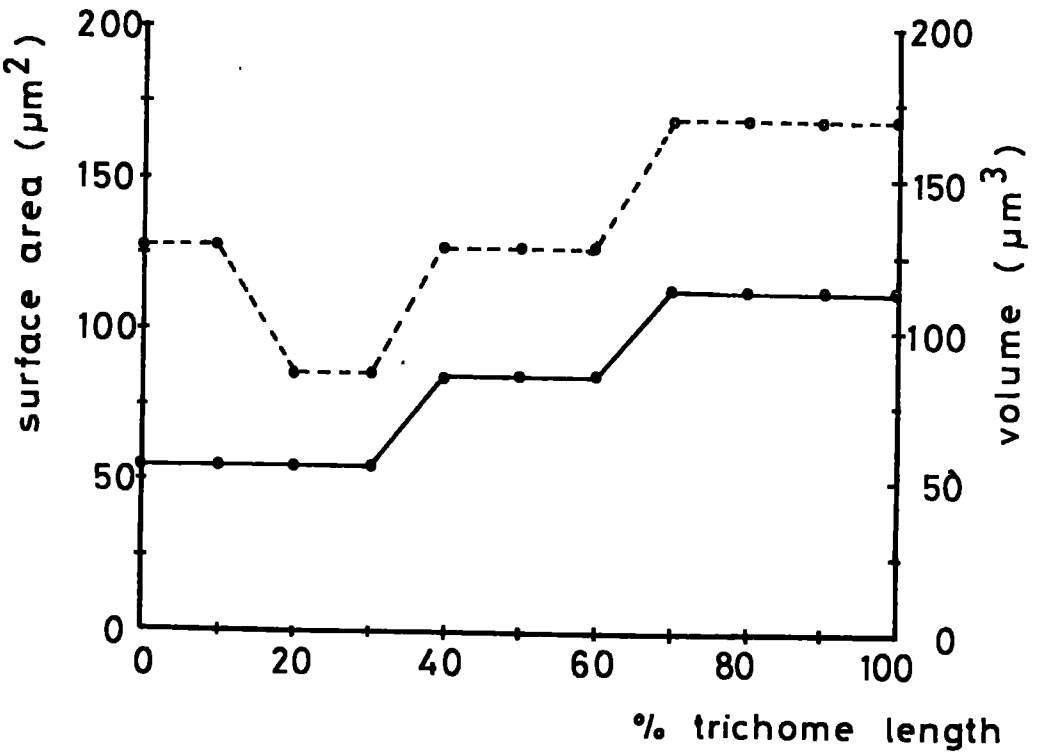

Calothrix brevissima
surface area (μm²)
volume (μm³)
150
100
50
0
0
20
40
60
80
100
% trichome length


Calothrix desertica
surface area (μm²)
volume (μm³)
200
150
100
50
0
0
20
40
60
80
100
% trichome length

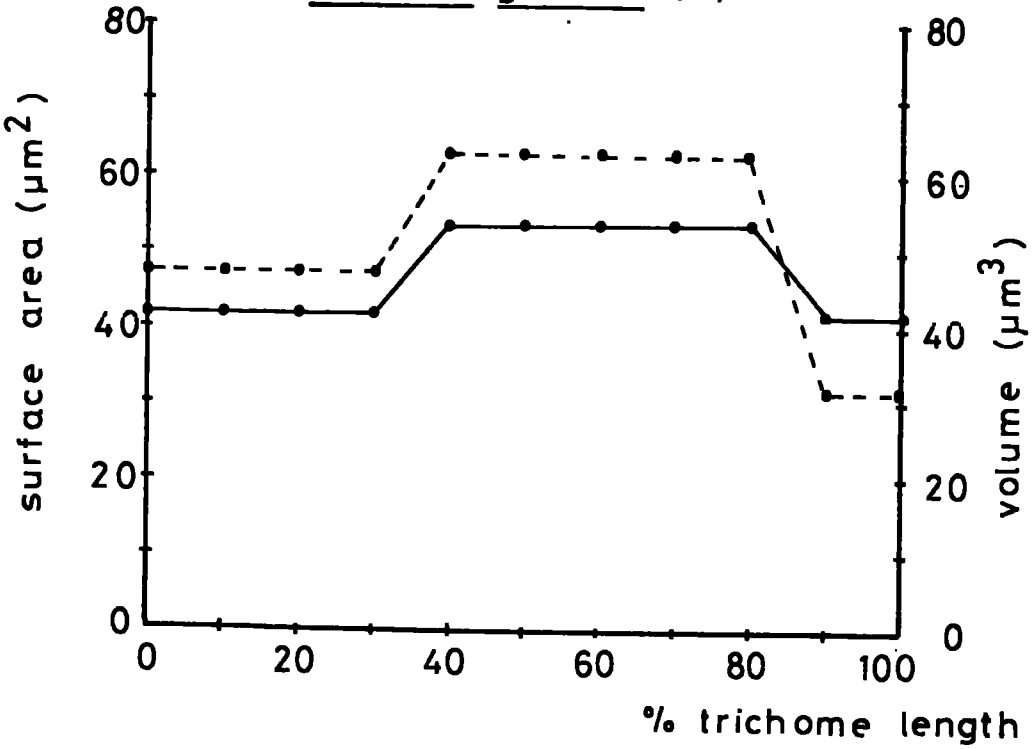
Calothrix fusca (a)



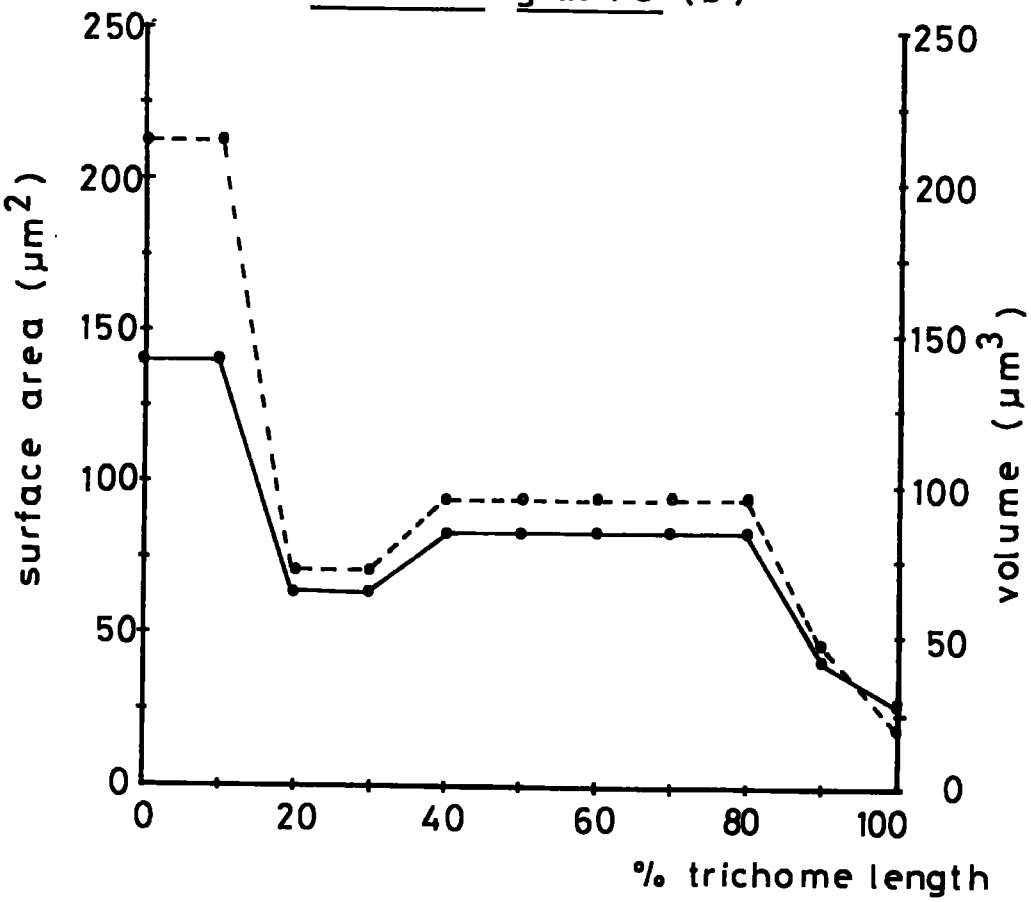
Calothrix fusca (b)

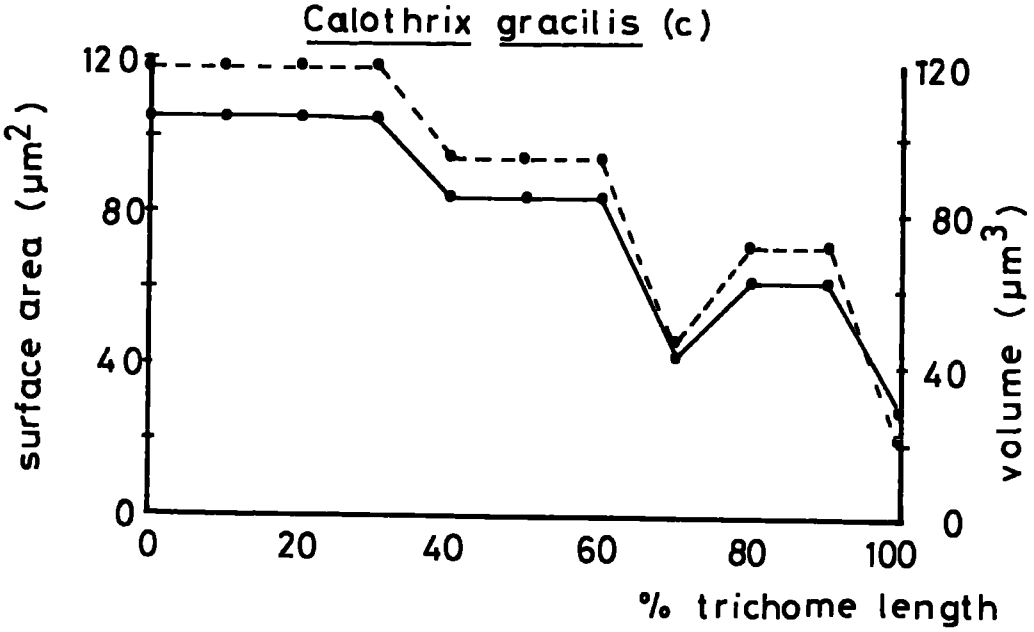


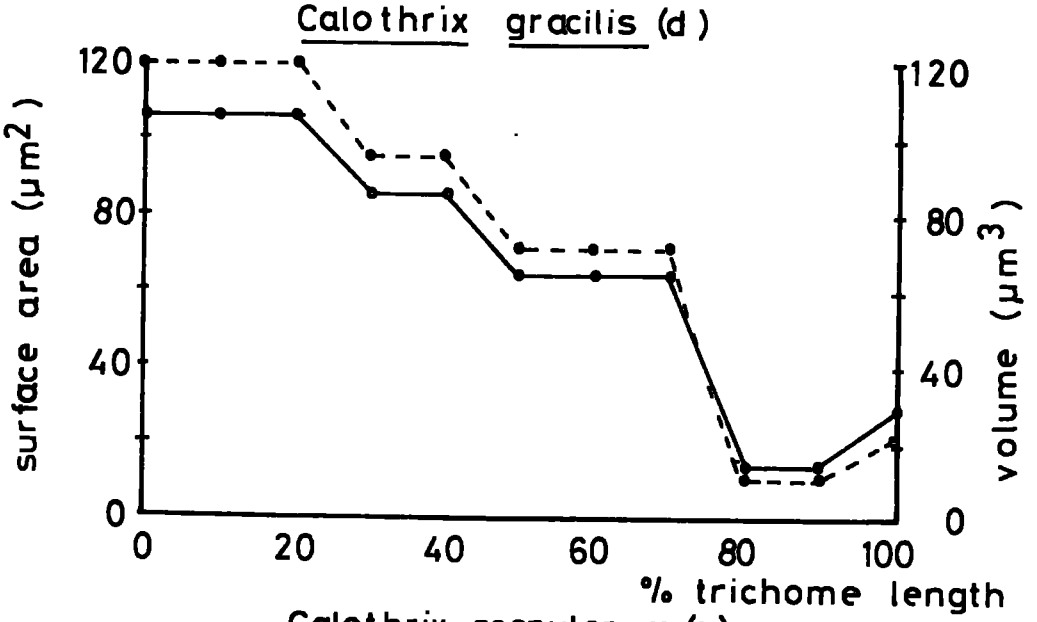
Calothrix gracilis (a)

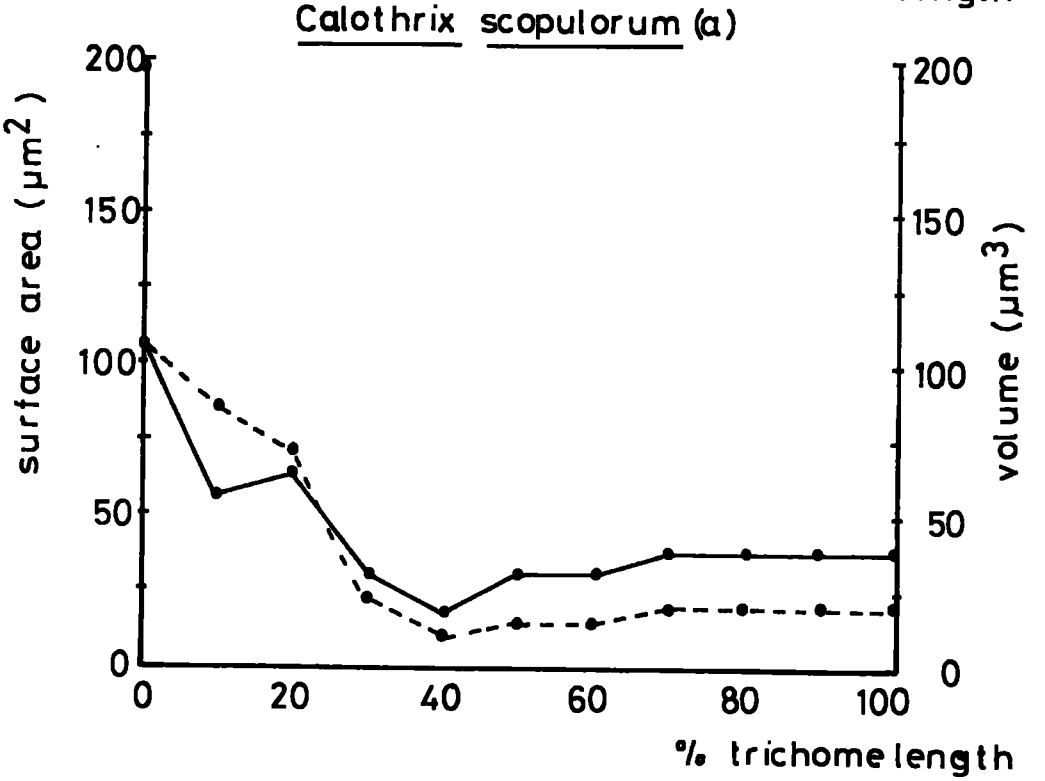


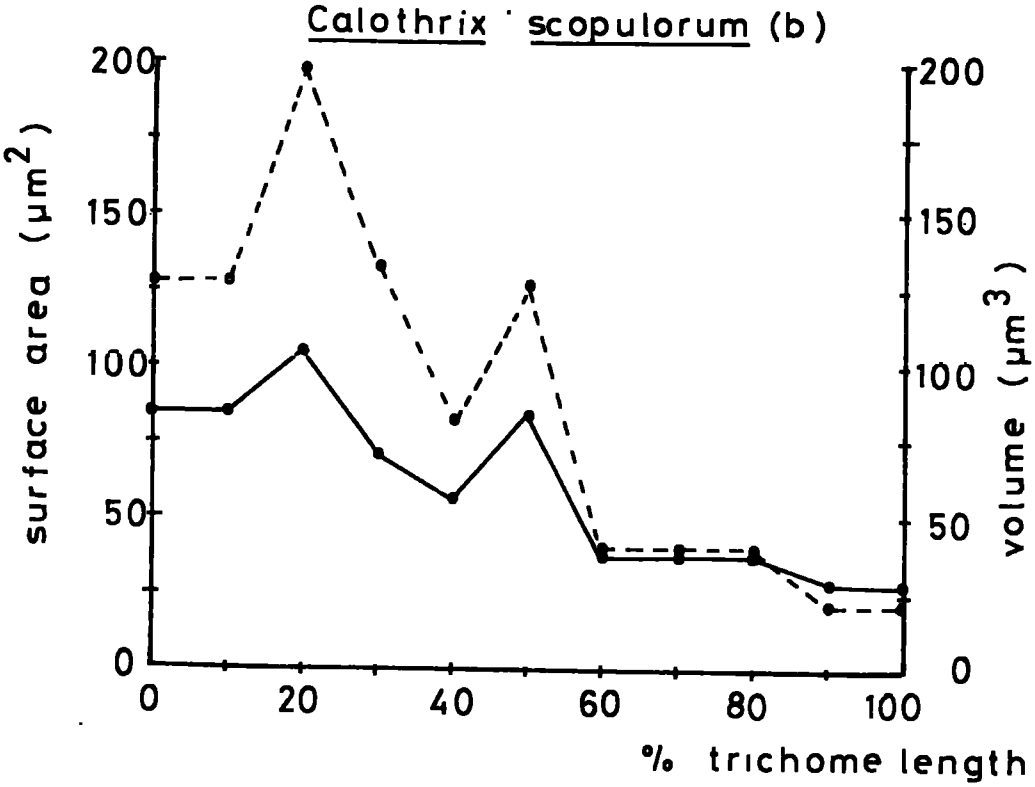
Calothrix gracilis (b)

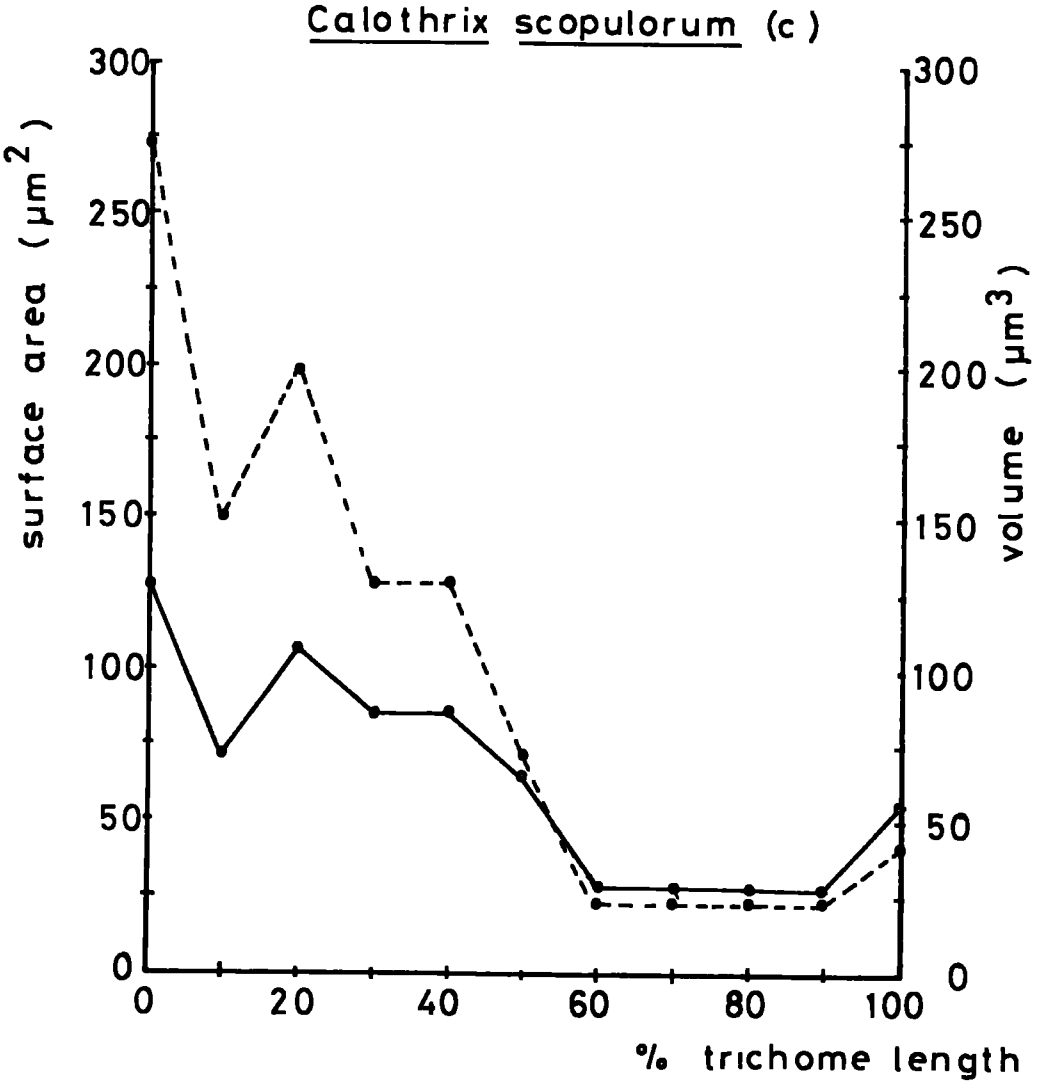



Calothrix gracilis (c)
surface area (µm²)
volume (µm³)
120
80
40
0
0
20
40
60
80
100
% trichome length

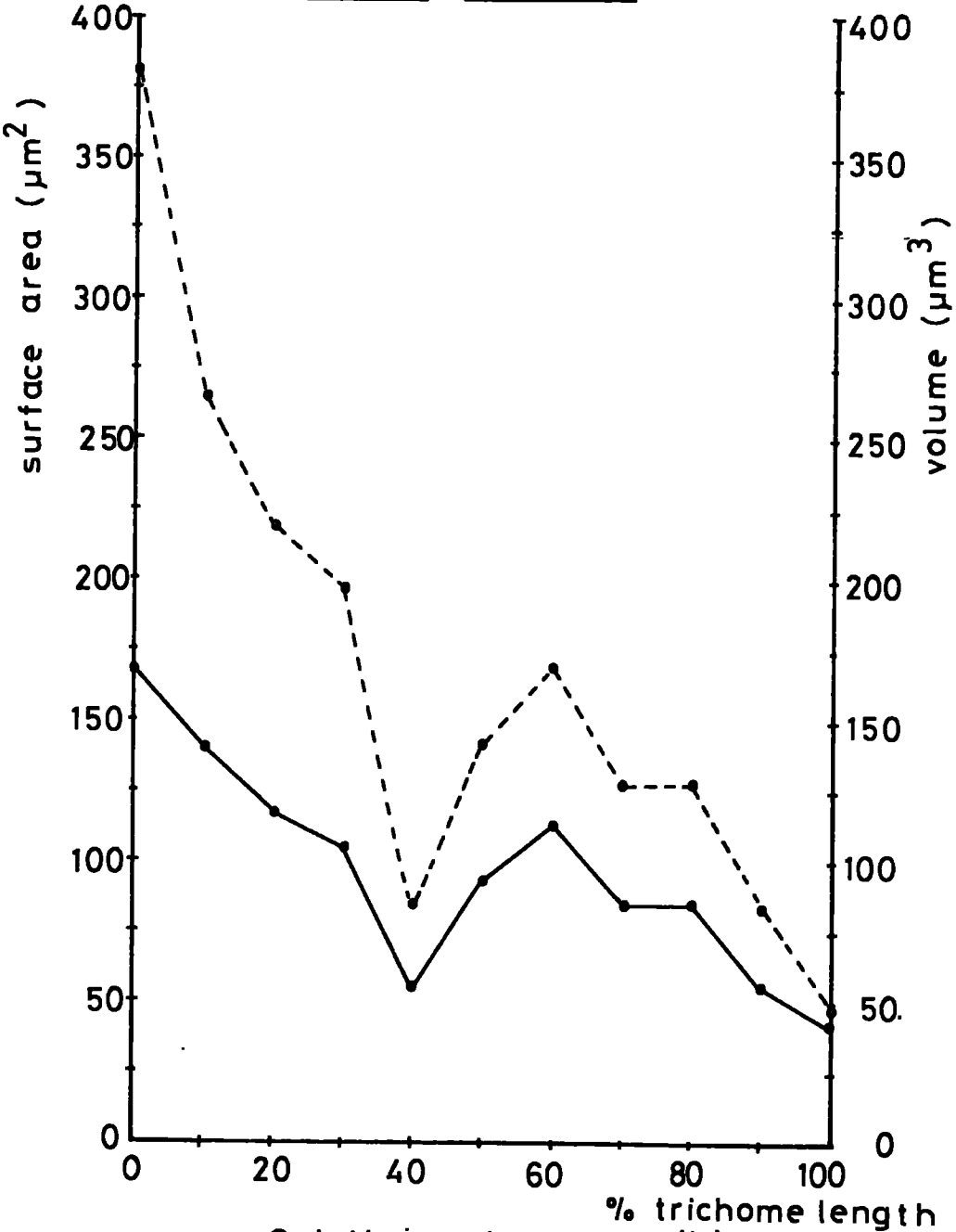

Calothrix gracilis (d)
surface area (µm²)
volume (µm³)
120
80
40
0
0
20
40
60
80
100
% trichome length


Calothrix scopulorum (a)
surface area (µm²)
volume (µm³)
200
150
100
50
0
0
20
40
60
80
100
% trichome length

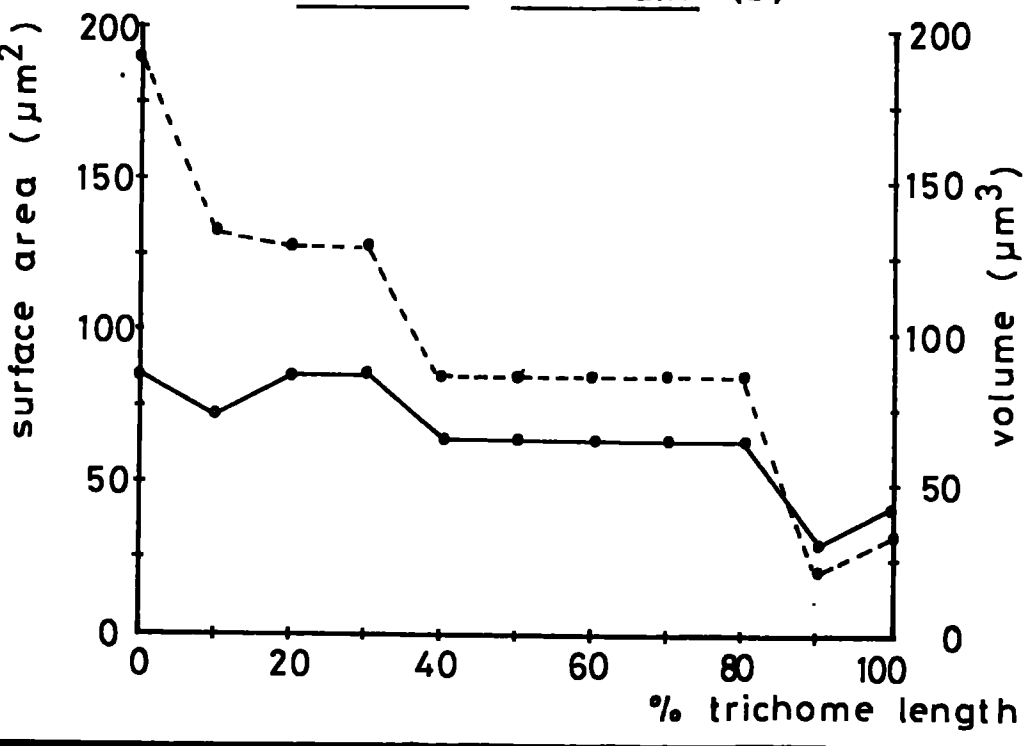

Calothrix scopulorum (b)
surface area (μm²)
volume (μm³)
200
150
100
50
0
0
20
40
60
80
100
% trichome length


Calothrix scopulorum (c)
surface area (μm²)
volume (μm³)
300
250
200
150
100
50
0
0
20
40
60
80
100
% trichome length

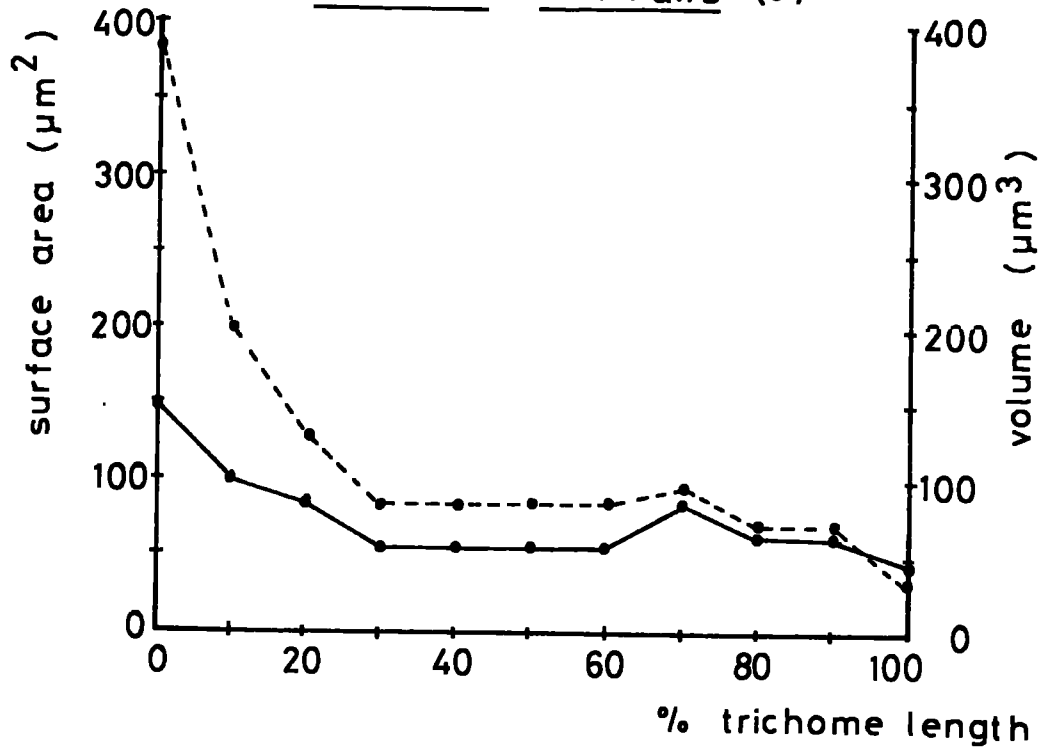
Calothrix thermalis (a)



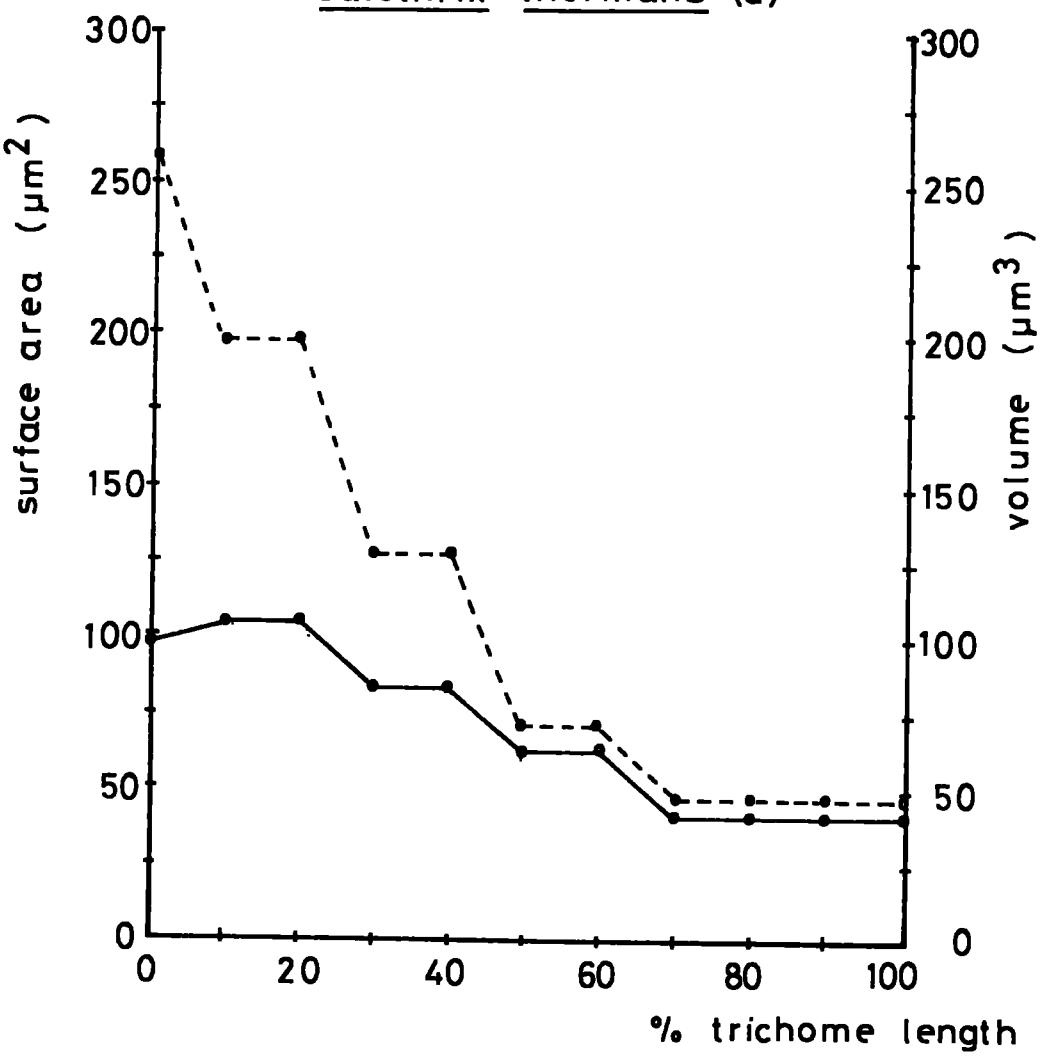
Calothrix thermalis (b)



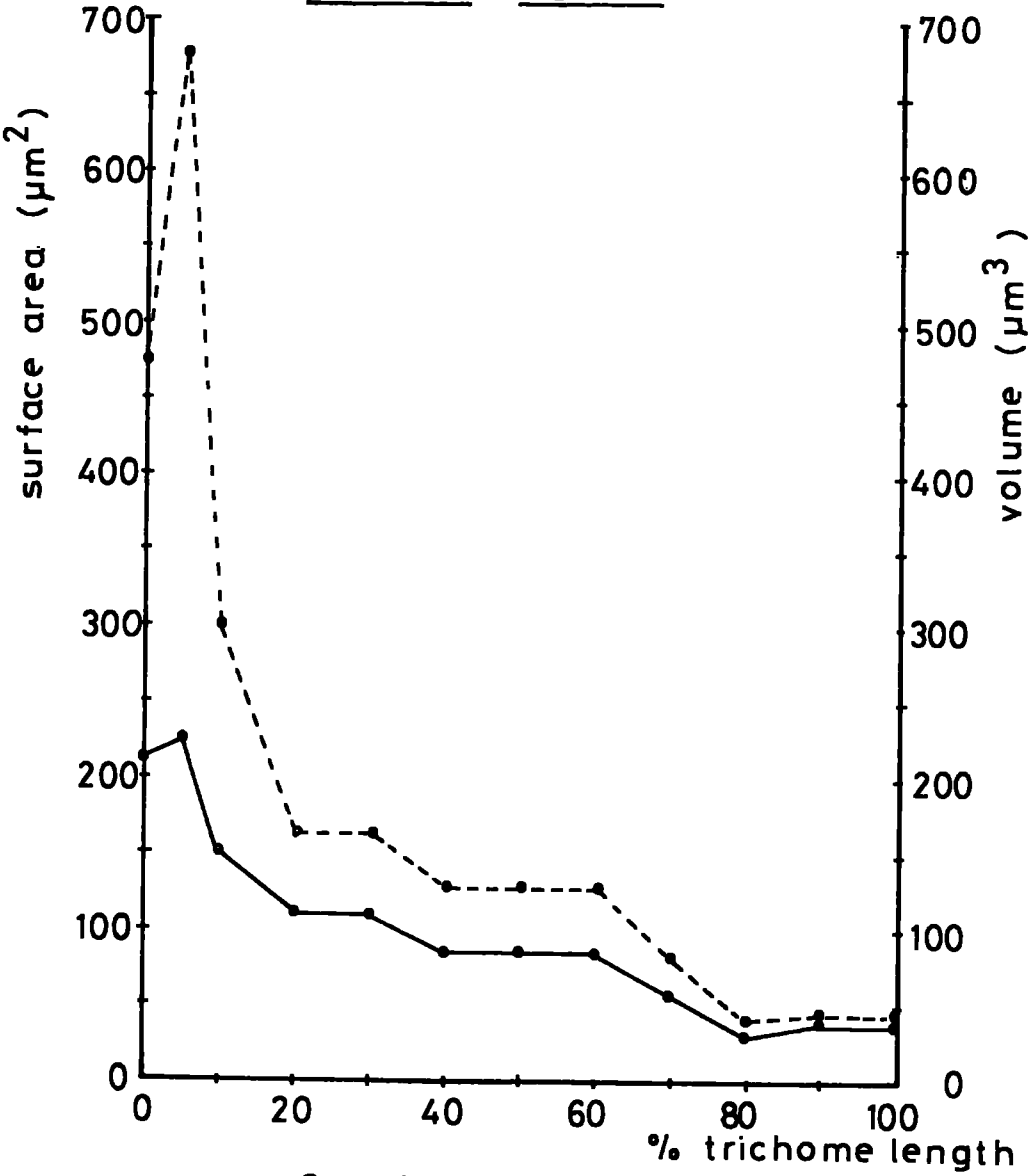
Calothrix thermalis (c)



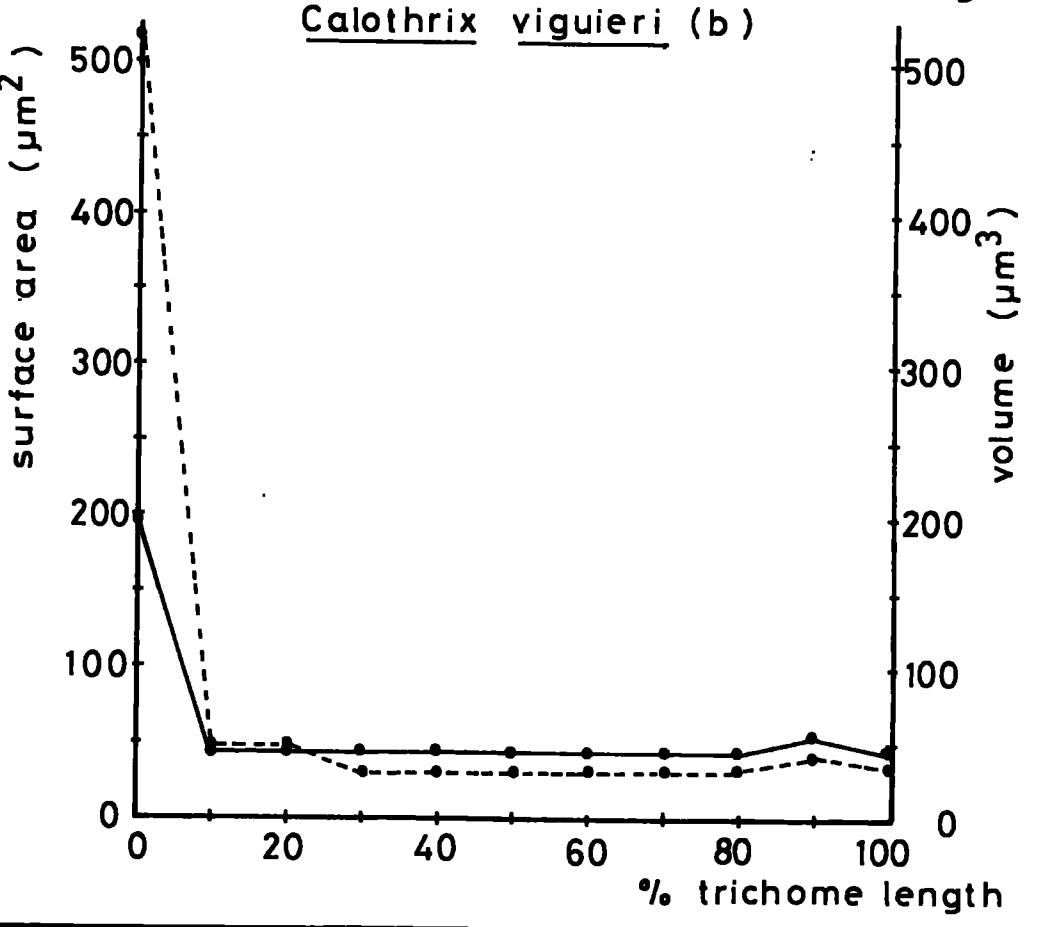
Calothrix thermalis (d)



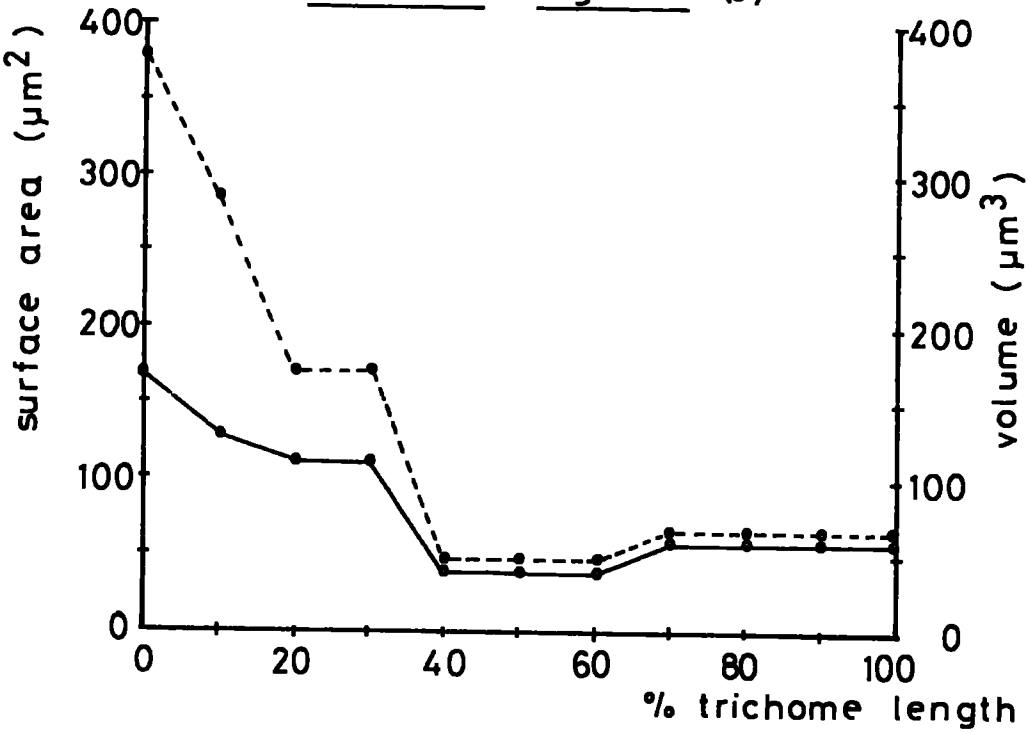
Calothrix viguieri (a)



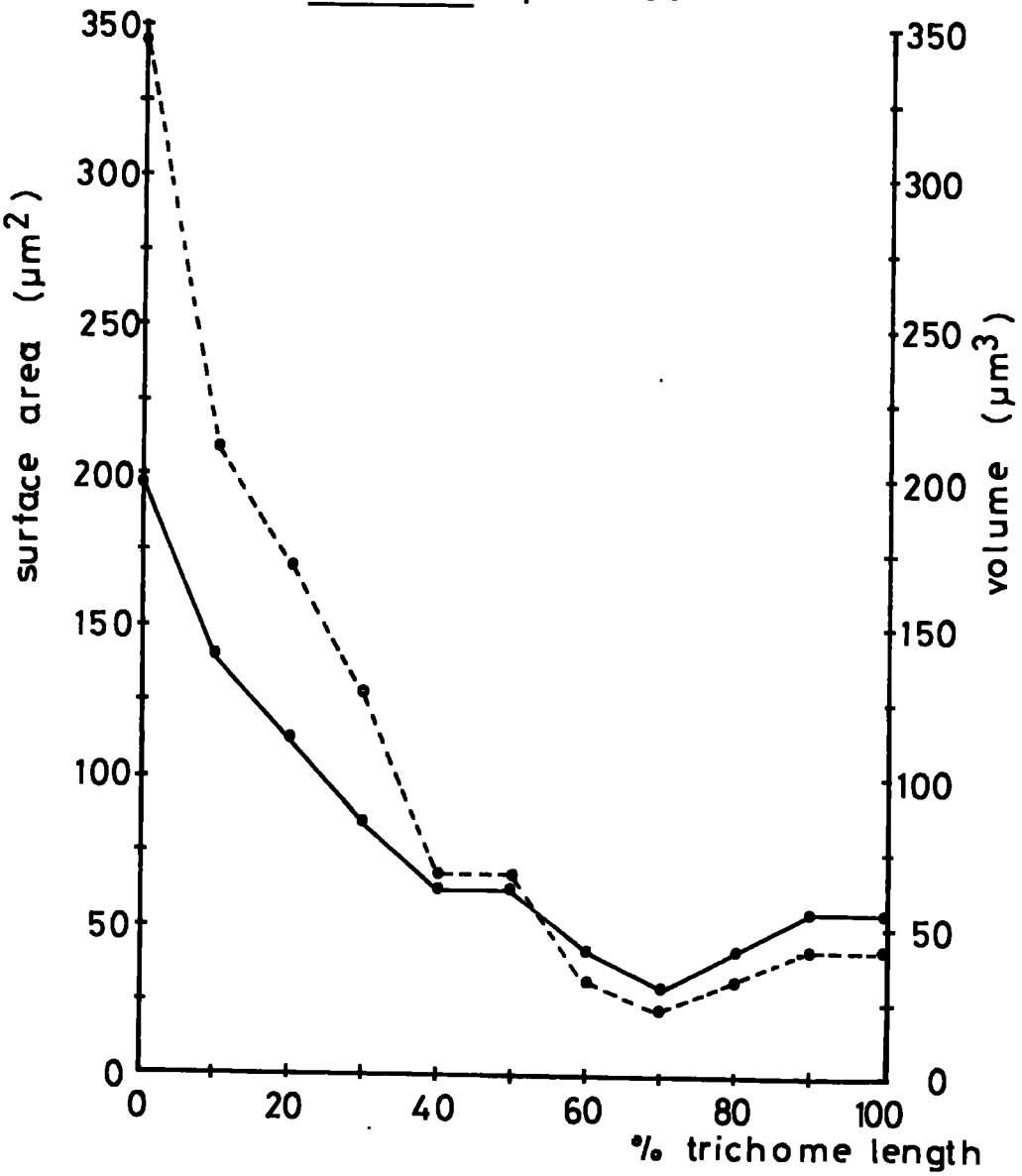
Calothrix viguieri (b)

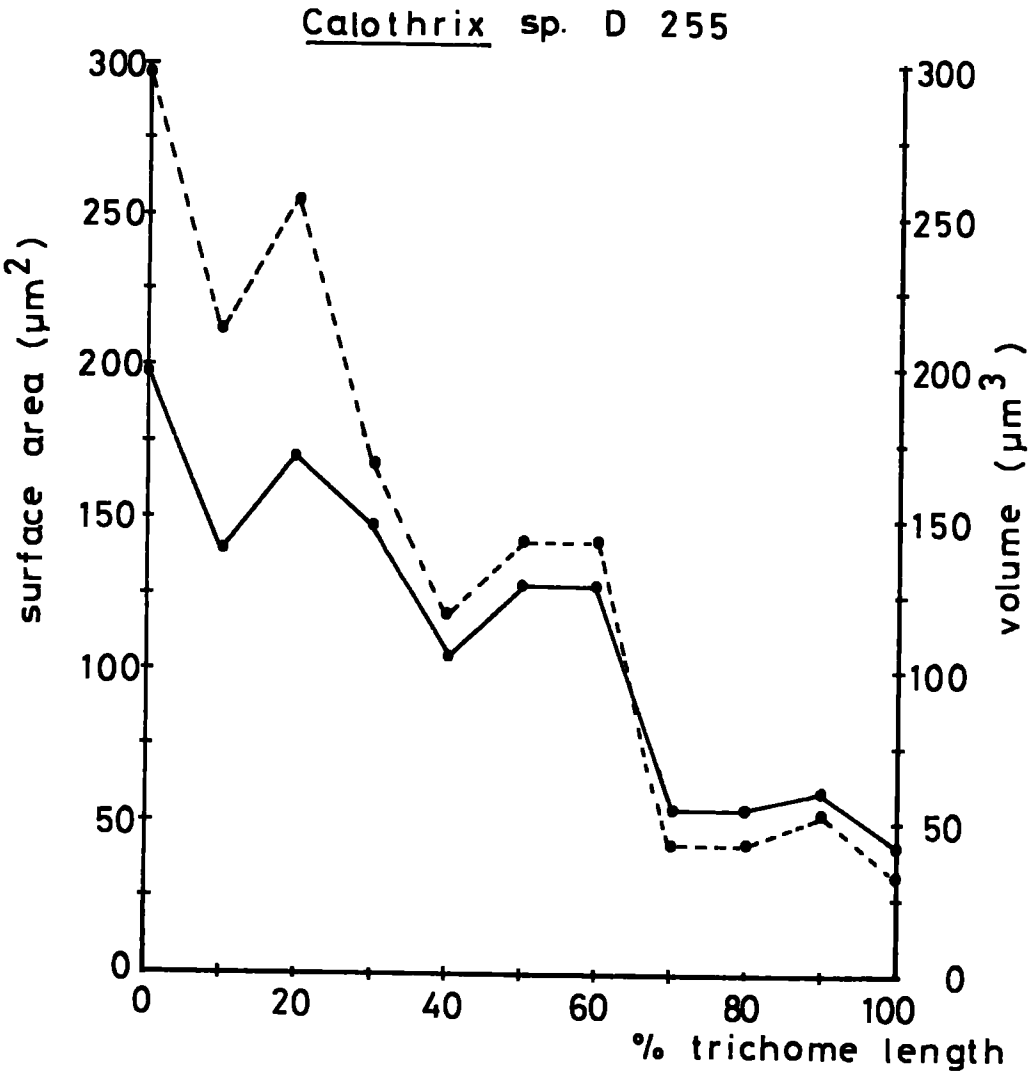


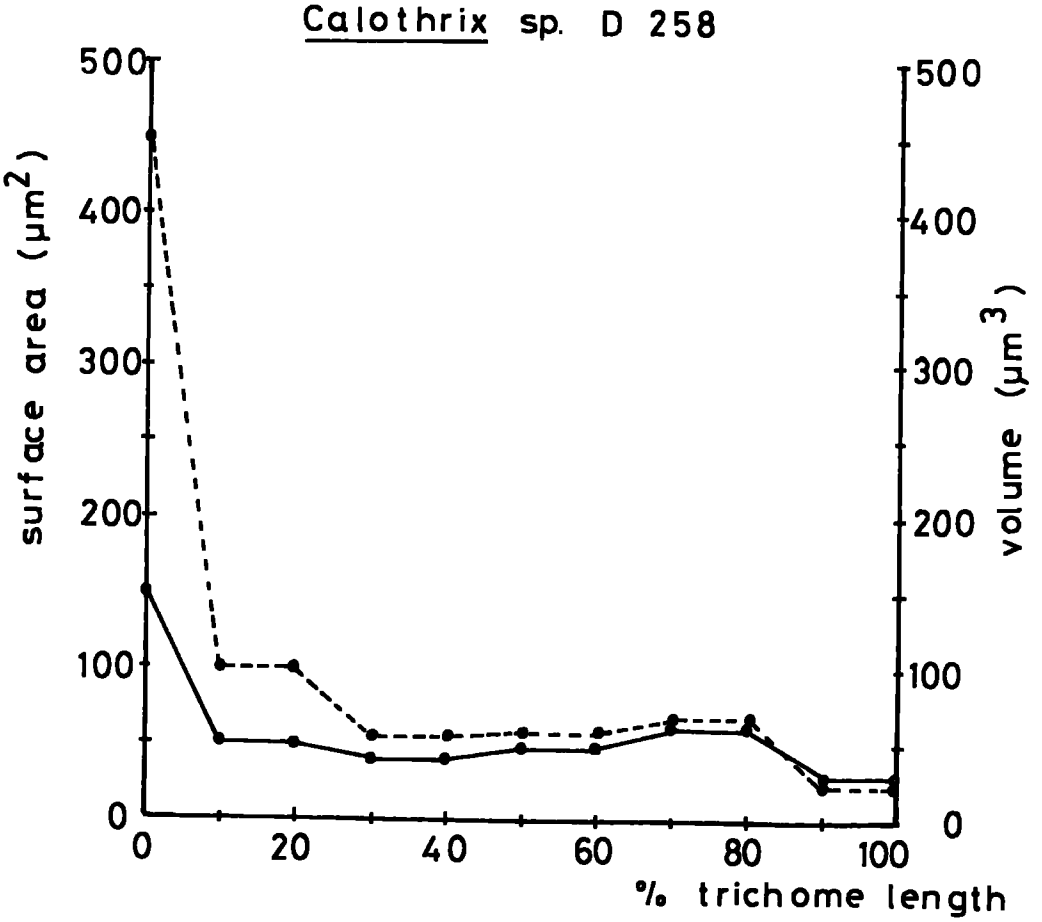
Calothrix viguieri (c)



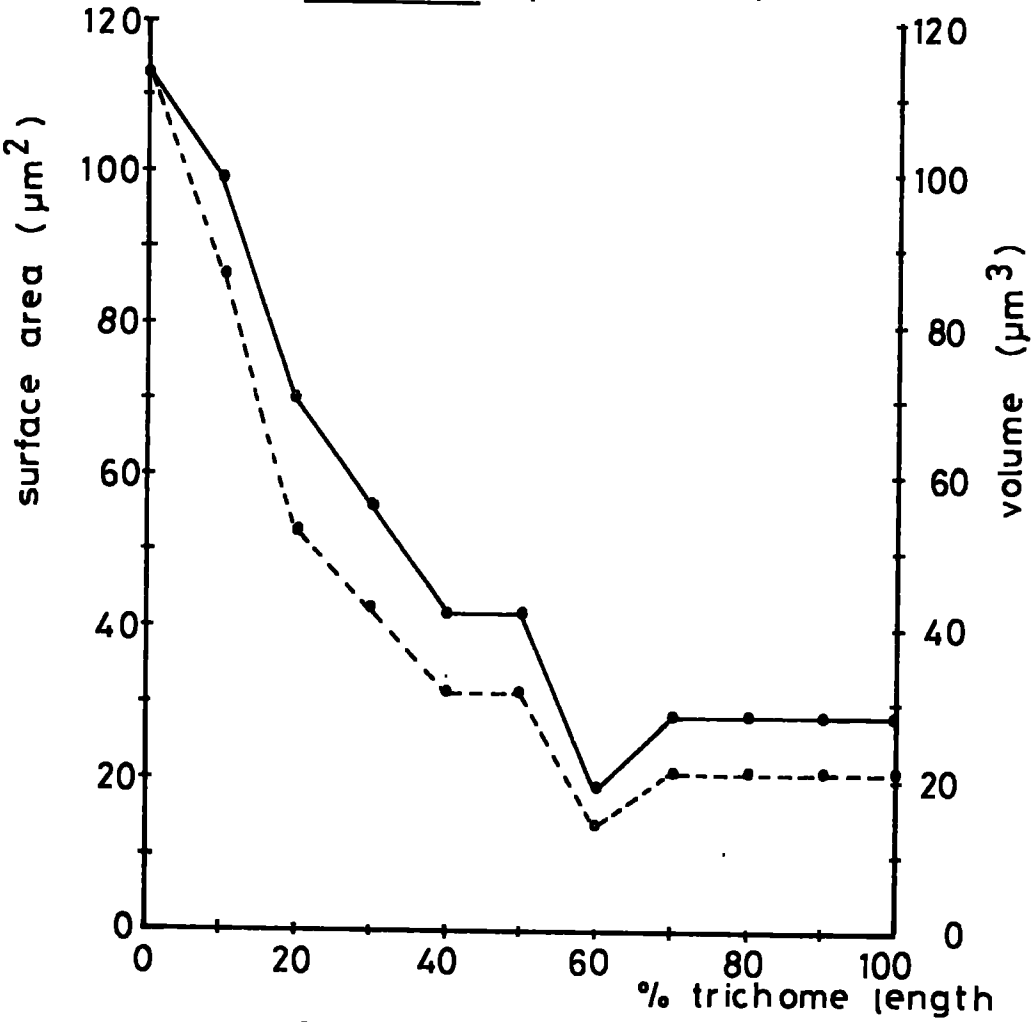
Calothrix sp. D 255



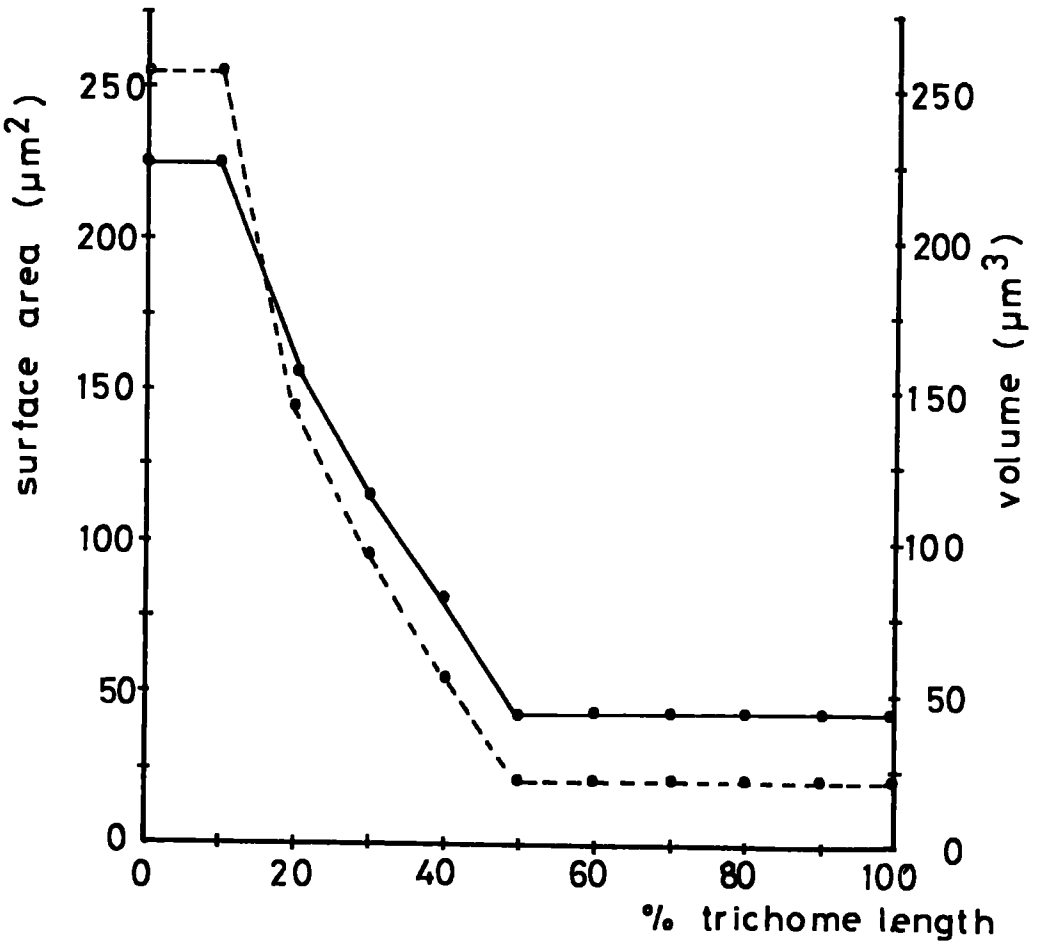

Calothrix sp. D 255
surface area (μm²)
volume (μm³)
% trichome length
0
50
100
150
200
250
300
0
20
40
60
80
100

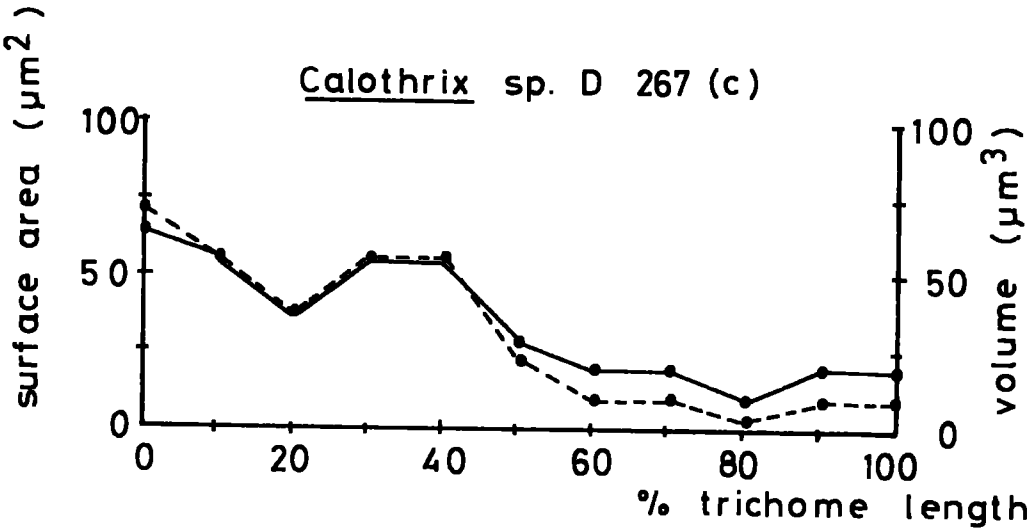

Calothrix sp. D 258
surface area (μm²)
volume (μm³)
% trichome length
0
100
200
300
400
500
0
20
40
60
80
100

Calothrix sp. D 267 (a)



Calothrix sp. D 267 (b)




Calothrix sp. D 267 (c)
surface area (μm²)
volume (μm³)
100
50
0
0
20
40
60
80
100
% trichome length

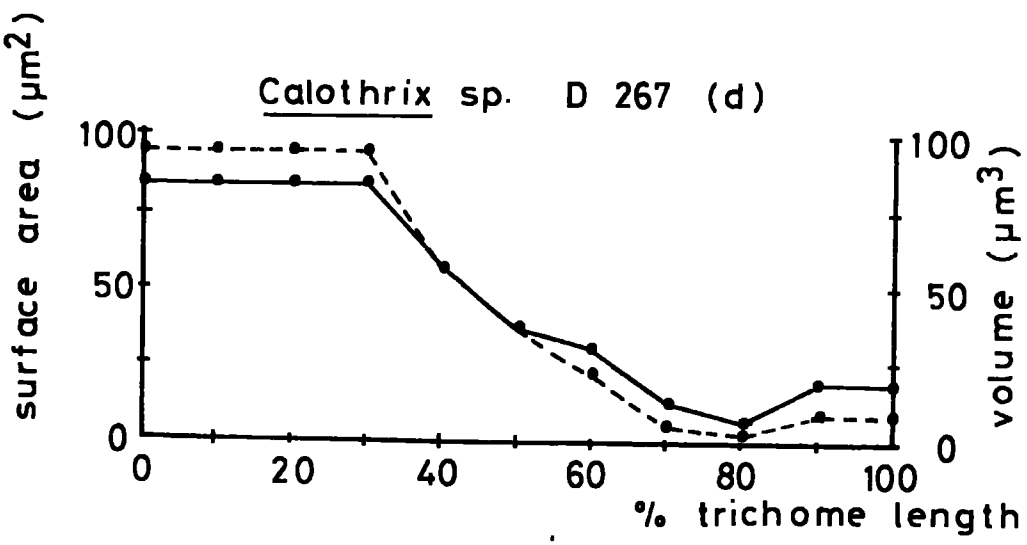

Calothrix sp. D 267 (d)
surface area (μm²)
volume (μm³)
100
50
0
0
20
40
60
80
100
% trichome length

Table 3.7 External surface area:volume relationships

species	basal cell	apical cell
Calothrix brevissima	0.60 : 1	0.69 : 1
C. elenkinii	0.53 : 1	1.05 : 1
C. fusca	0.40 : 1	0.78 : 1
C. gracilis	0.85 : 1	0.99 : 1
C. scopulorum	0.63 : 1	1.64 : 1
C. thermalis	0.39 : 1	0.77 : 1
C. viguieri	0.37 : 1	0.73 : 1
Calothrix sp. D255	0.64 : 1	0.71 : 1
Calothrix sp. D 258	0.73 : 1	1.05 : 1
Calothrix sp. D 267	0.89 : 1	1.46 : 1
Gloeotrichia (Brasside)	0.53 : 1	1.06 : 1
Gloeotrichia (Sunbiggin)	0.39 : 1	0.88 : 1

For any detailed investigations concerning the influence of environmental factors on hair length, quantitative or at least semi-quantitative estimates will be essential. Hair length is probably the simplest quantitative measure. In the majority of specimens examined by the author, the loss of colour occurred over a very short portion of the trichome and the beginning of the hair could be distinguished easily. At the light microscope level the loss in colour appears to be associated with a loss in pigment or with the development of a vacuolate appearance. (Fig. 3.7). In the latter case, pigment may still be visible, but may be confined to the walls of the cells. (Difficulties in measuring hair length can arise if there is no such 'transition zone' and pigment is lost gradually).

Hairs were observed in laboratory culture (C. scopulorum) on one occasion (Section 3.4). Changes in the dimensions of normal vegetative cells and hair cells of this species and several field Rivulariaceae, are shown in Fig. 3.8.

It is evident that there is a marked elongation of the apical hair cells, the external surface area being greater than that of the other cells in the trichome in several cases. Plotting hair length against total trichome length (Fig. 3.9) shows some tendency for the length of the hair to increase as the trichome length increases.

3.4 Hairs in laboratory culture

During this work, 11 species of Calothrix were observed in

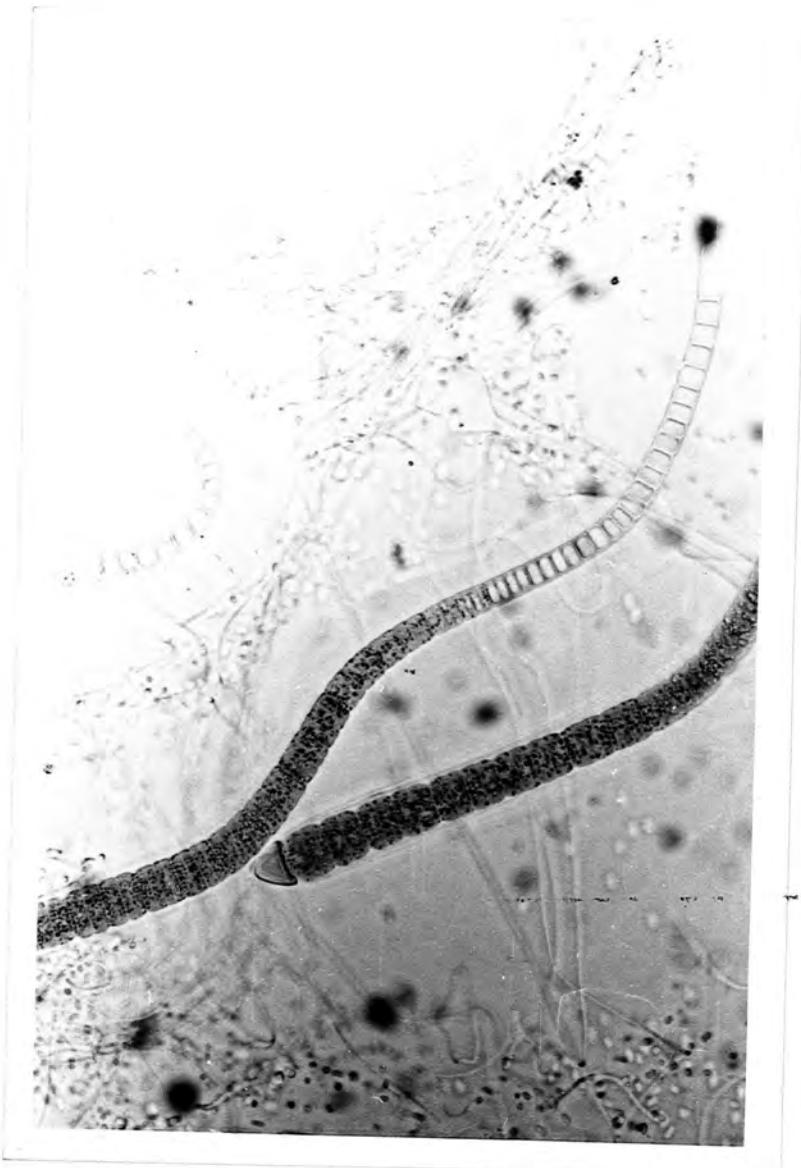


Fig. 3.7 *Rivularia* trichome with hair

Fig. 3.8 Changes in cell dimensions of trichomes with hairs

(a) *Calothrix scopulorum*

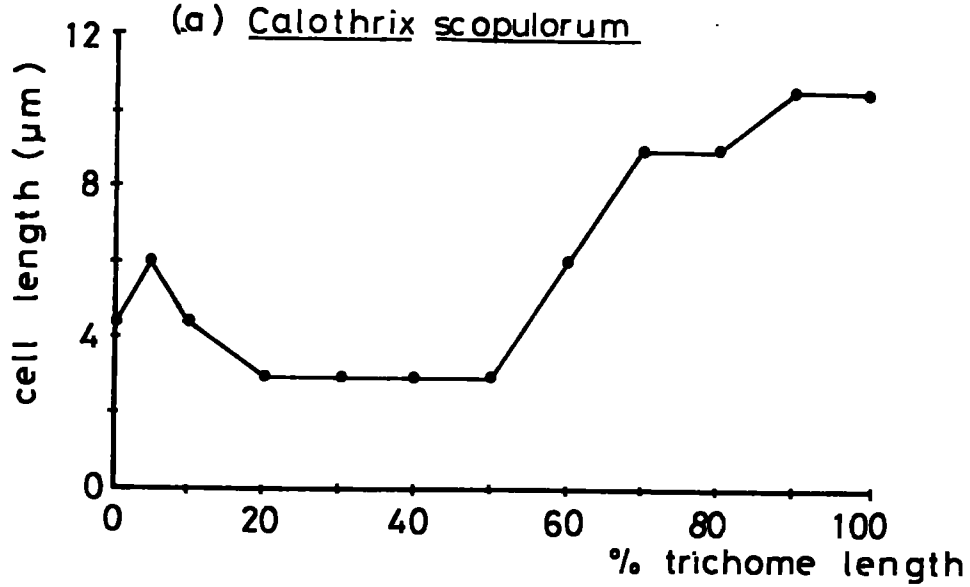
(b) *Gloeotrichia* (Brasside)

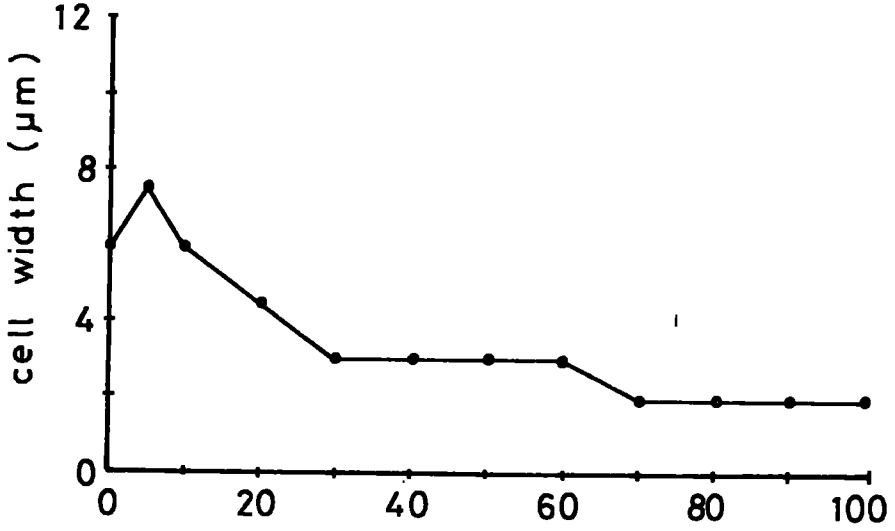
(c) *Gloeotrichia* (Llangorse)

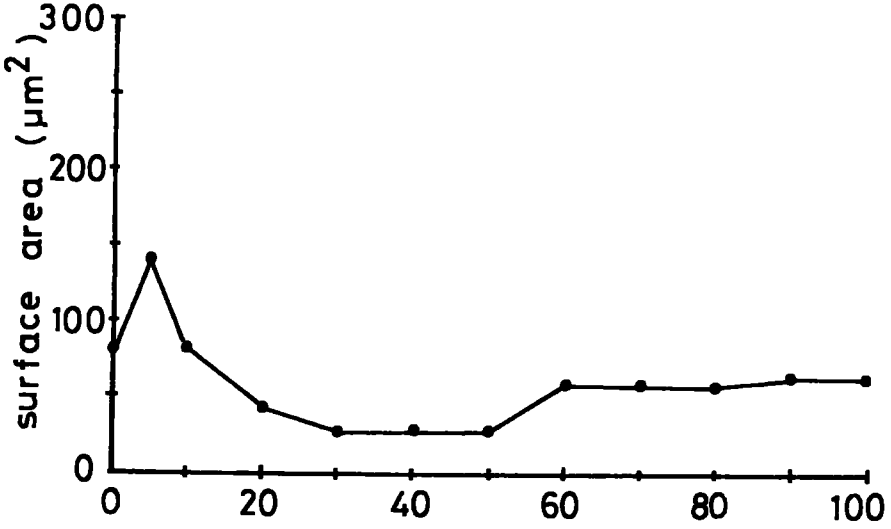
(d) *Gloeotrichia* (Sunbiggin)

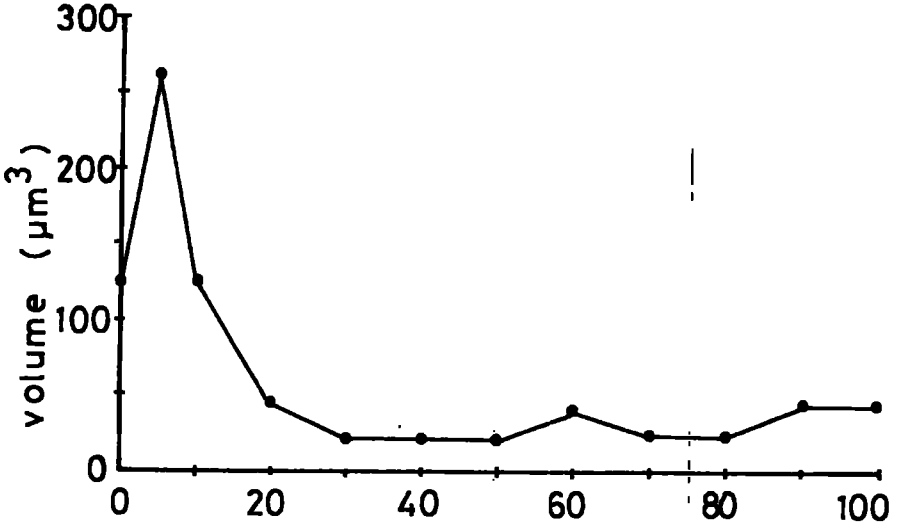
(e) *Rivularia* (Slapestone)

(f) *Rivularia* (Tulach hill)

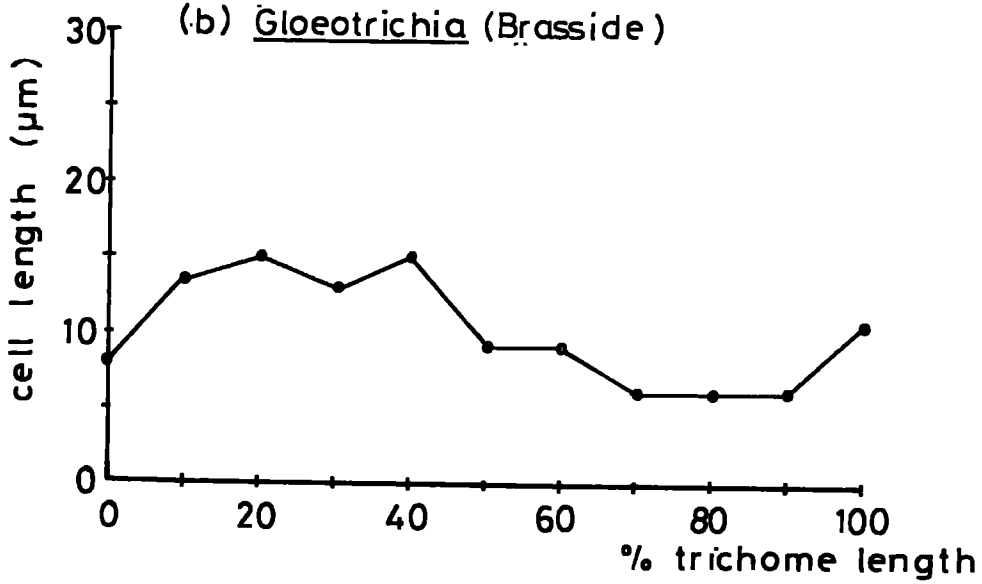

(a) Calothrix scopulorum
cell length (µm)
12
8
4
0
0
20
40
60
80
100
% trichome length

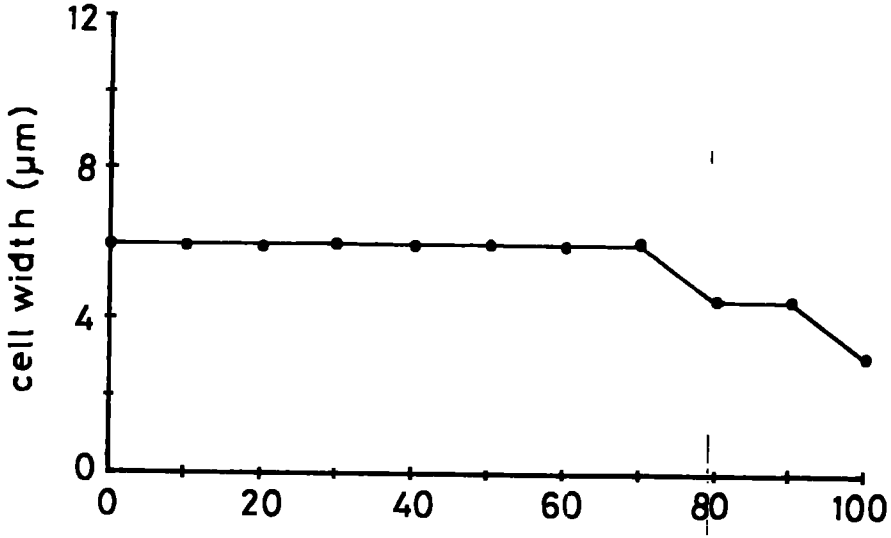

cell width (µm)
12
8
4
0
0
20
40
60
80
100

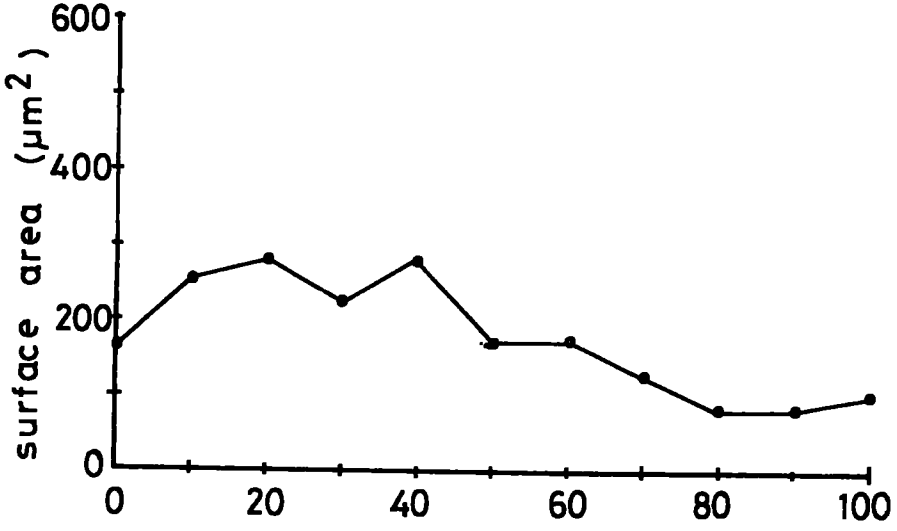

surface area (µm²)
300
200
100
0
0
20
40
60
80
100

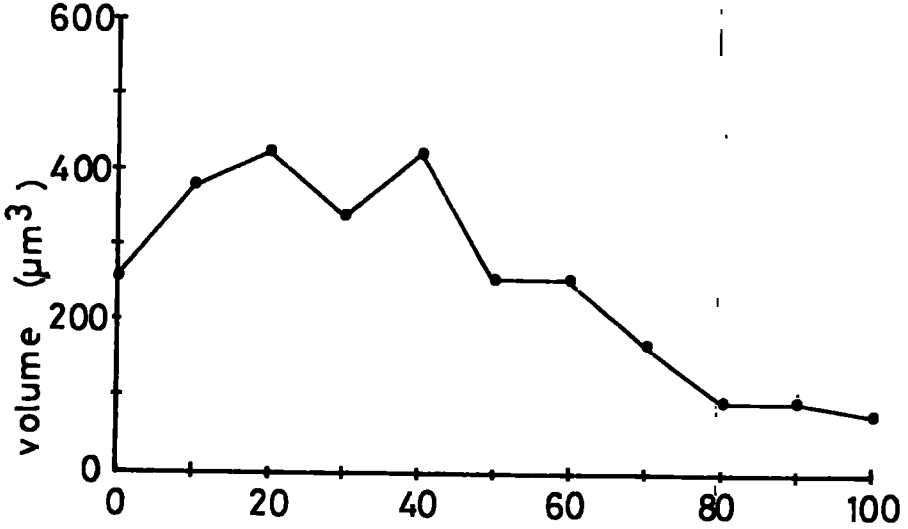

volume (µm³)
300
200
100
0
0
20
40
60
80
100

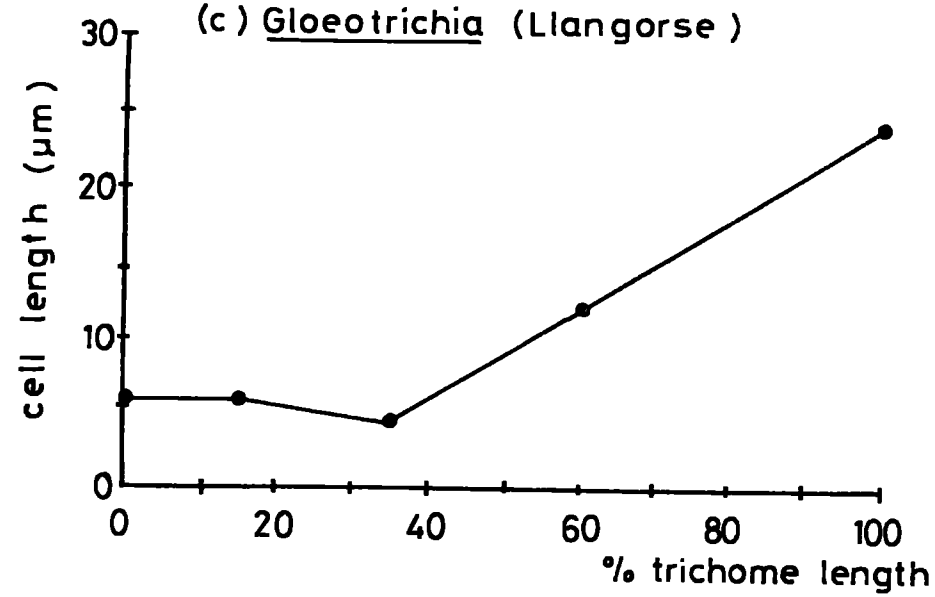
(b) Gloeotrichia (Brasside)

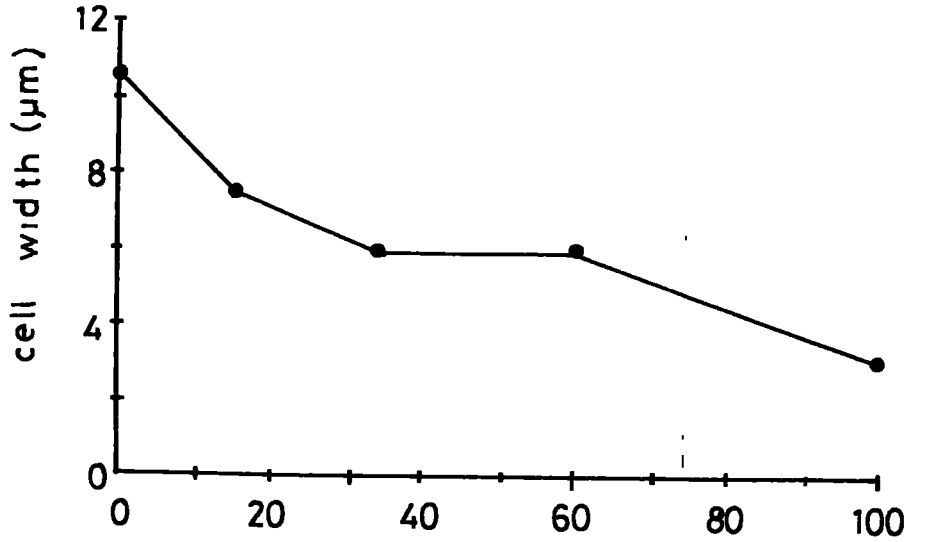


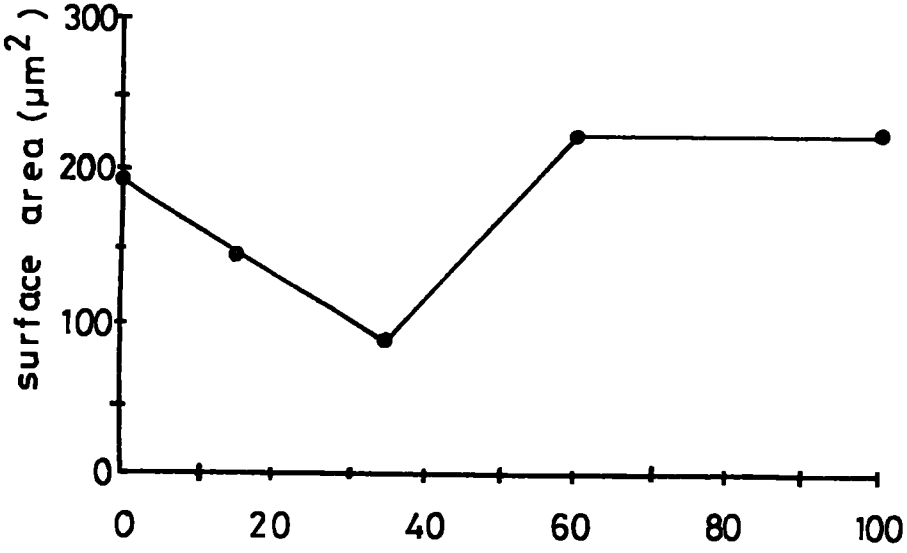


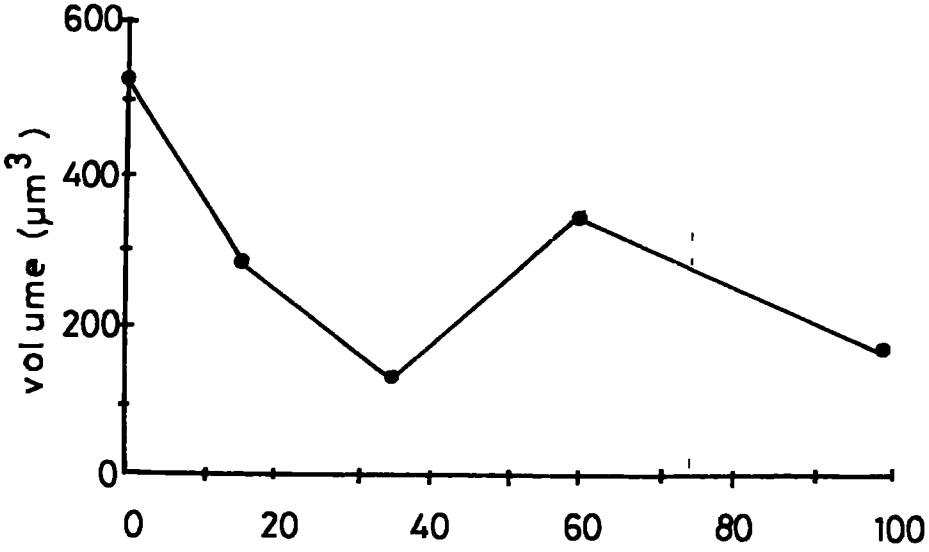




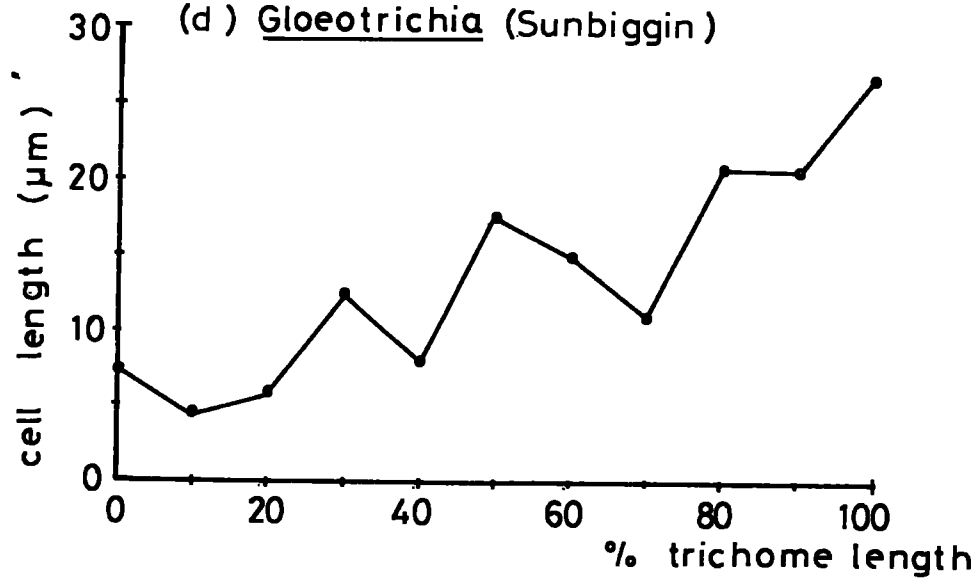

(c) Gloeotrichia (Llangorse)
cell length (µm)
30
20
10
0
0
20
40
60
80
100
% trichome length

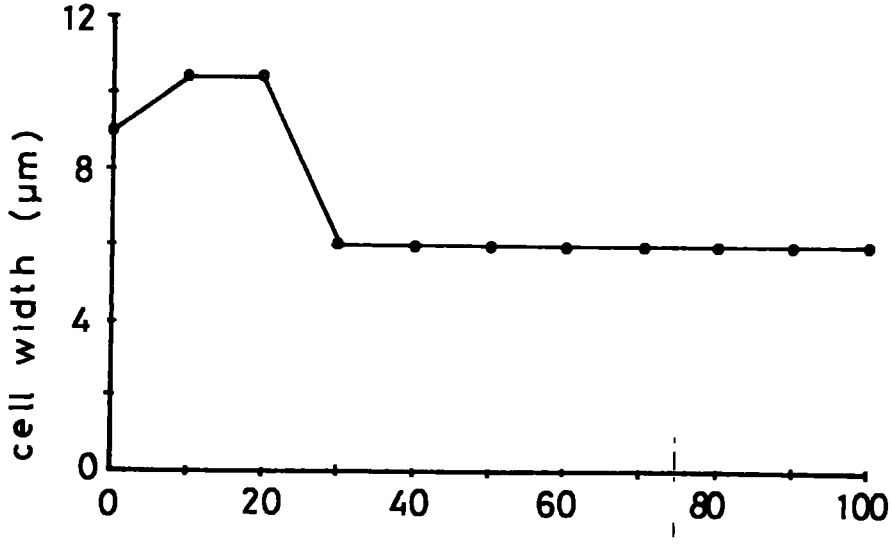

cell width (µm)
12
8
4
0
0
20
40
60
80
100

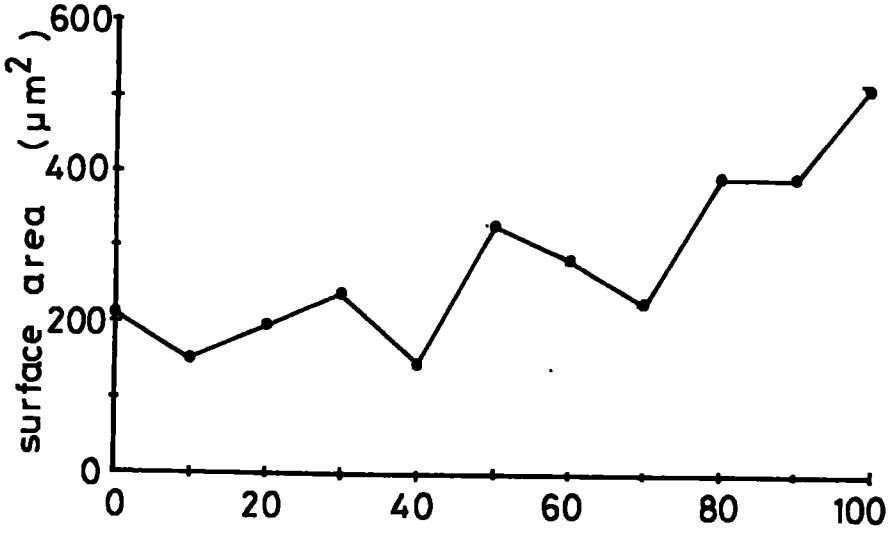

surface area (µm²)
300
200
100
0
0
20
40
60
80
100

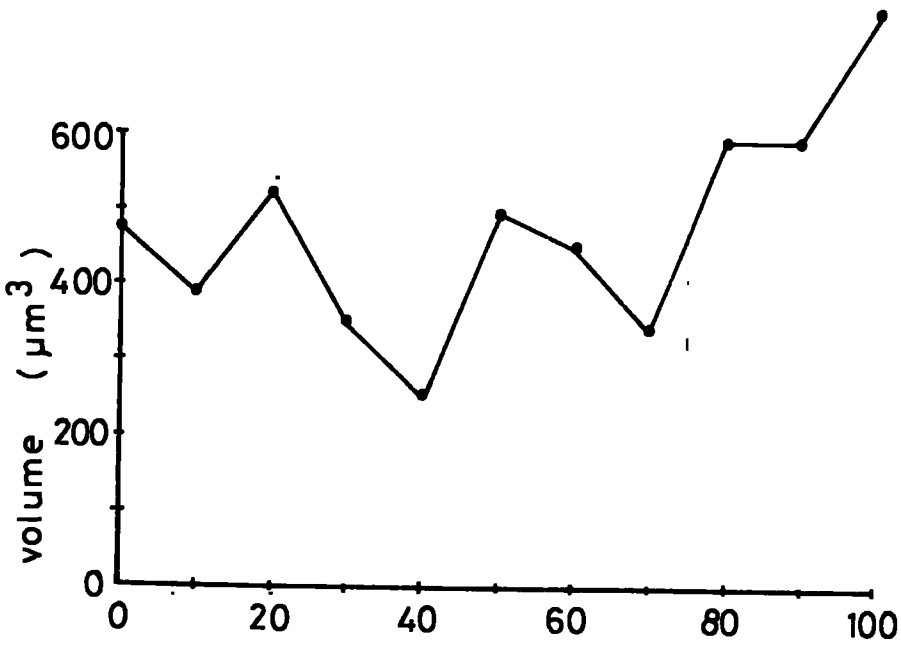

volume (µm³)
600
400
200
0
0
20
40
60
80
100

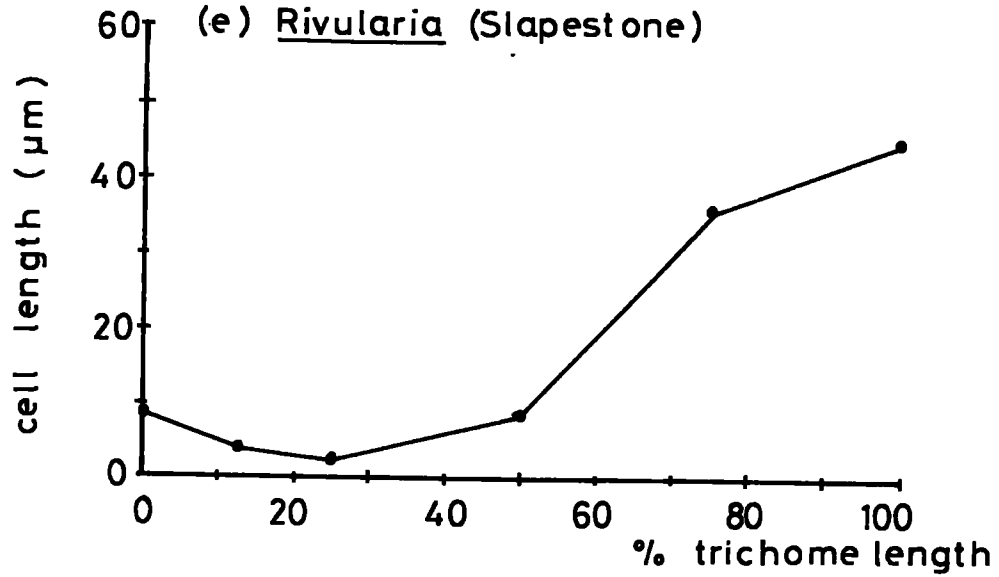
(d) Gloeotrichia (Sunbiggin)

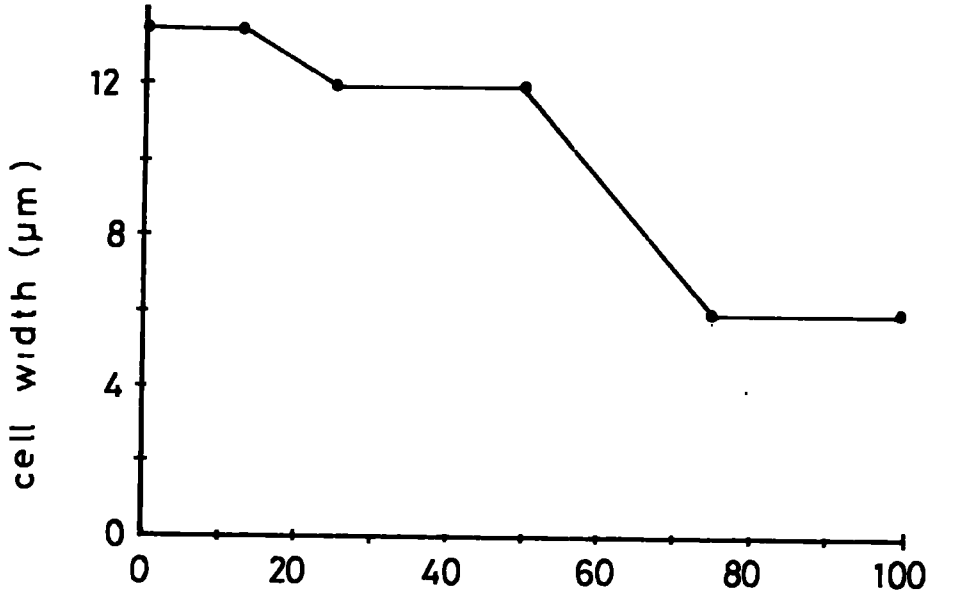


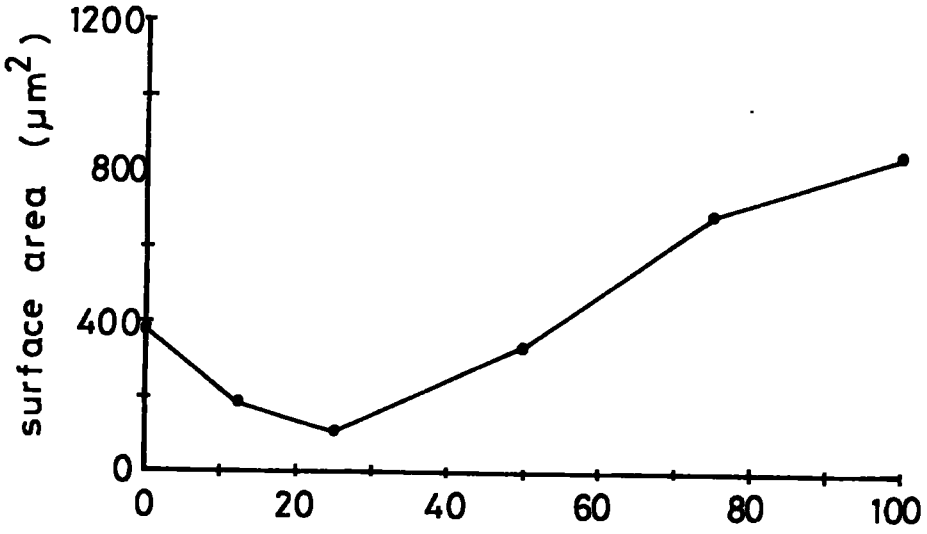


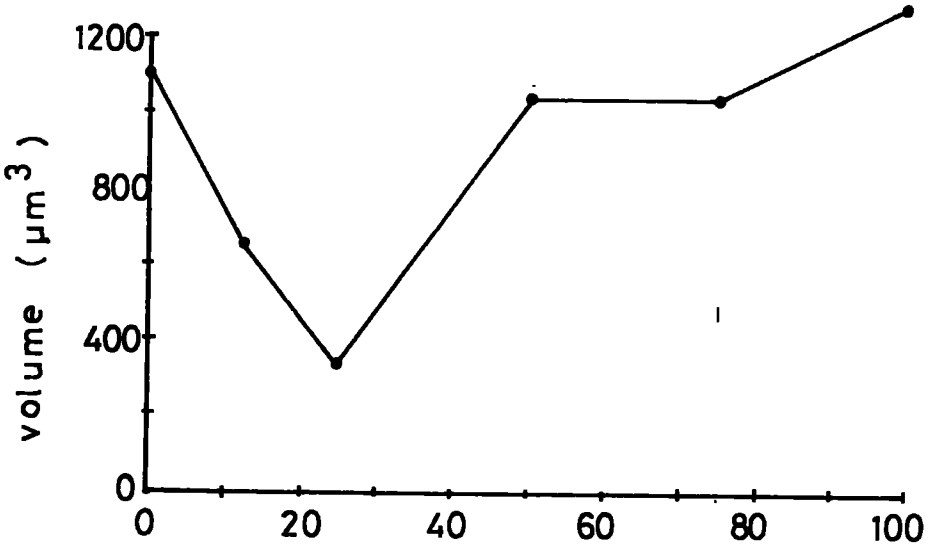




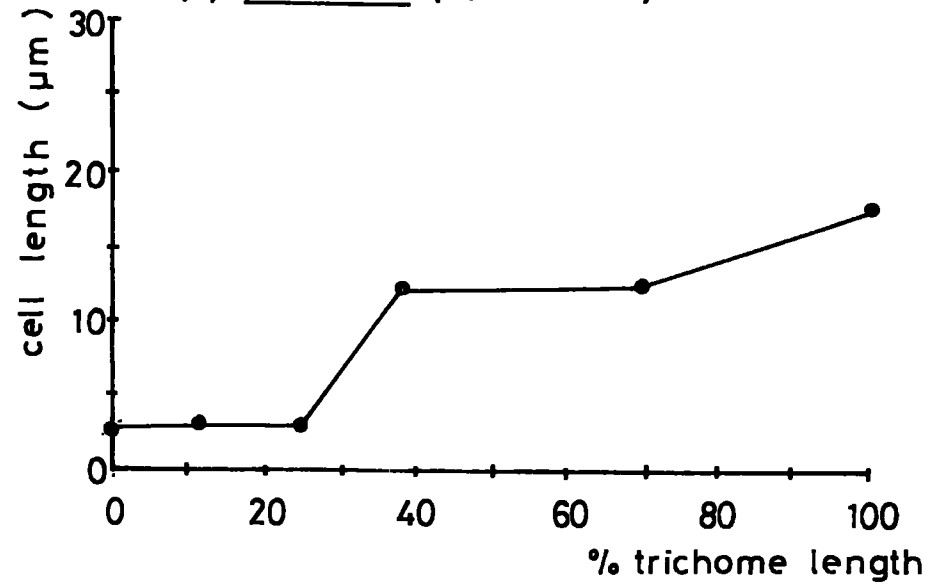

(e) Rivularia (Slapestone)
cell length (µm)
60
40
20
0
0
20
40
60
80
100
% trichome length

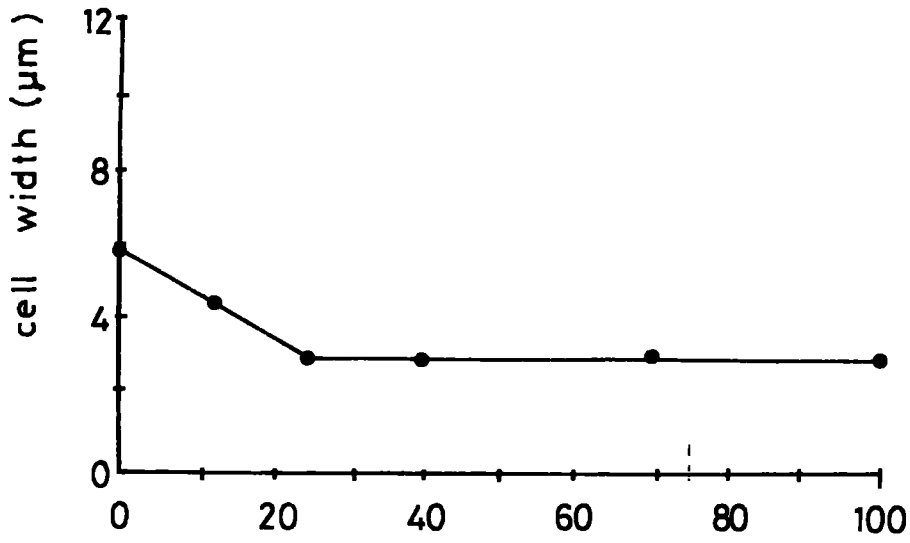

cell width (µm)
12
8
4
0
0
20
40
60
80
100

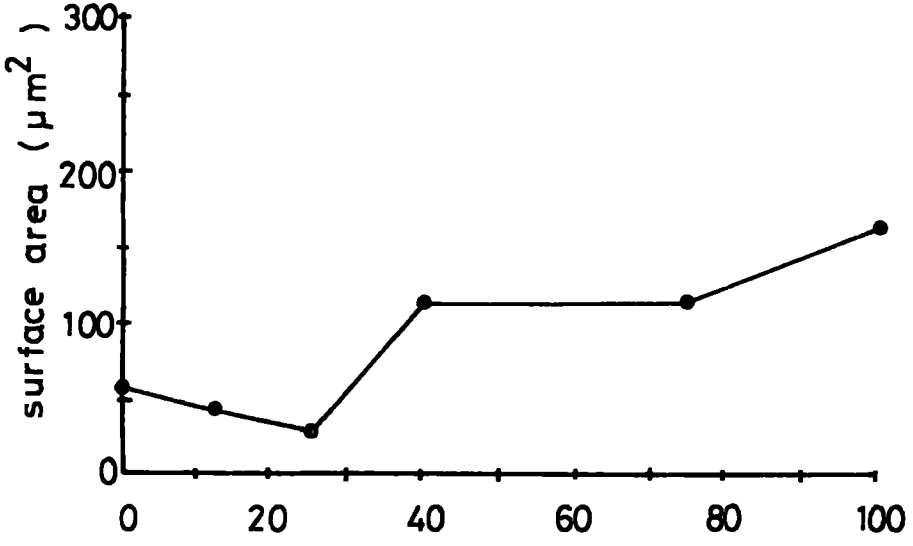

surface area (µm²)
1200
800
400
0
0
20
40
60
80
100

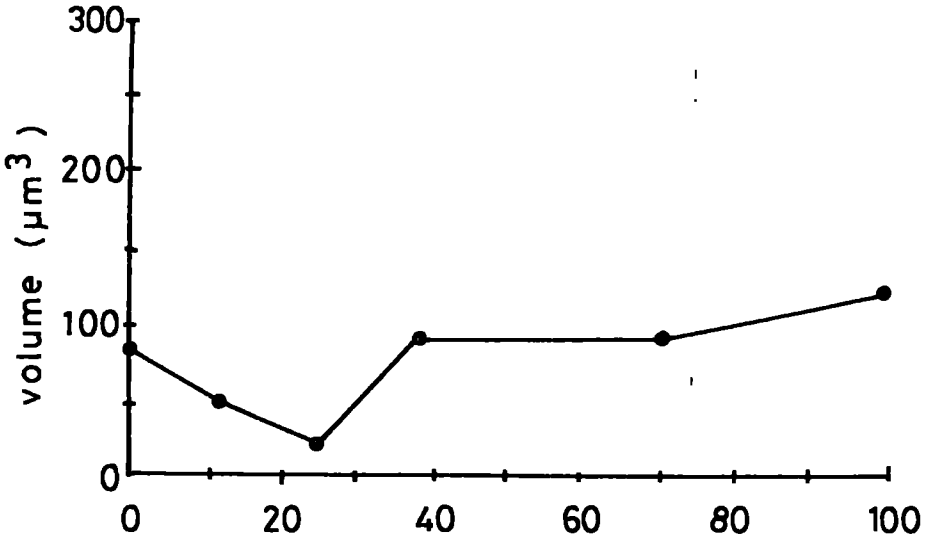

volume (µm³)
1200
800
400
0
0
20
40
60
80
100

(f) Rivularia (Tulach hill)









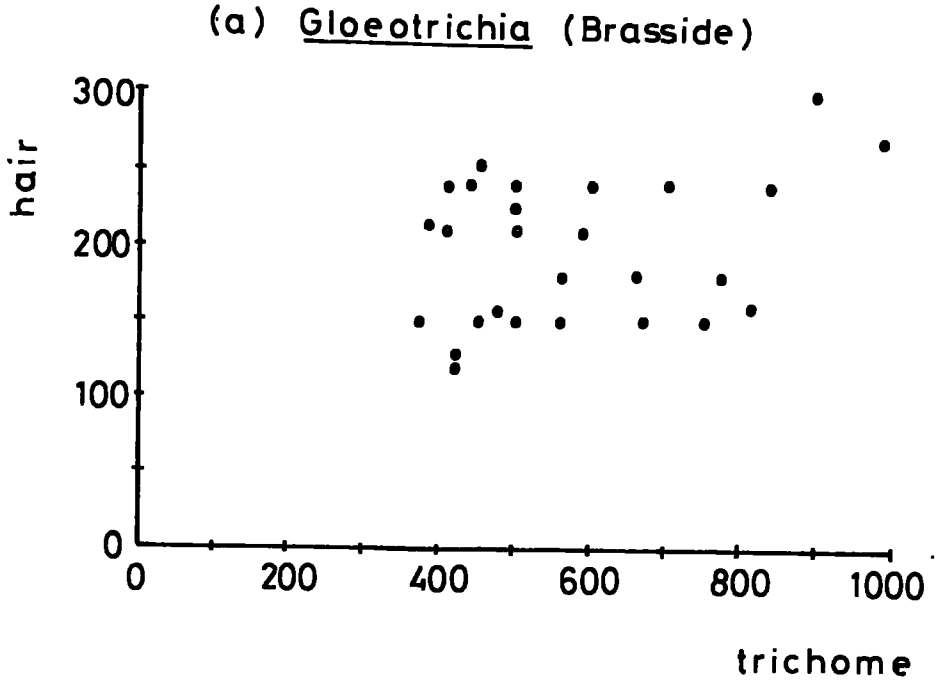
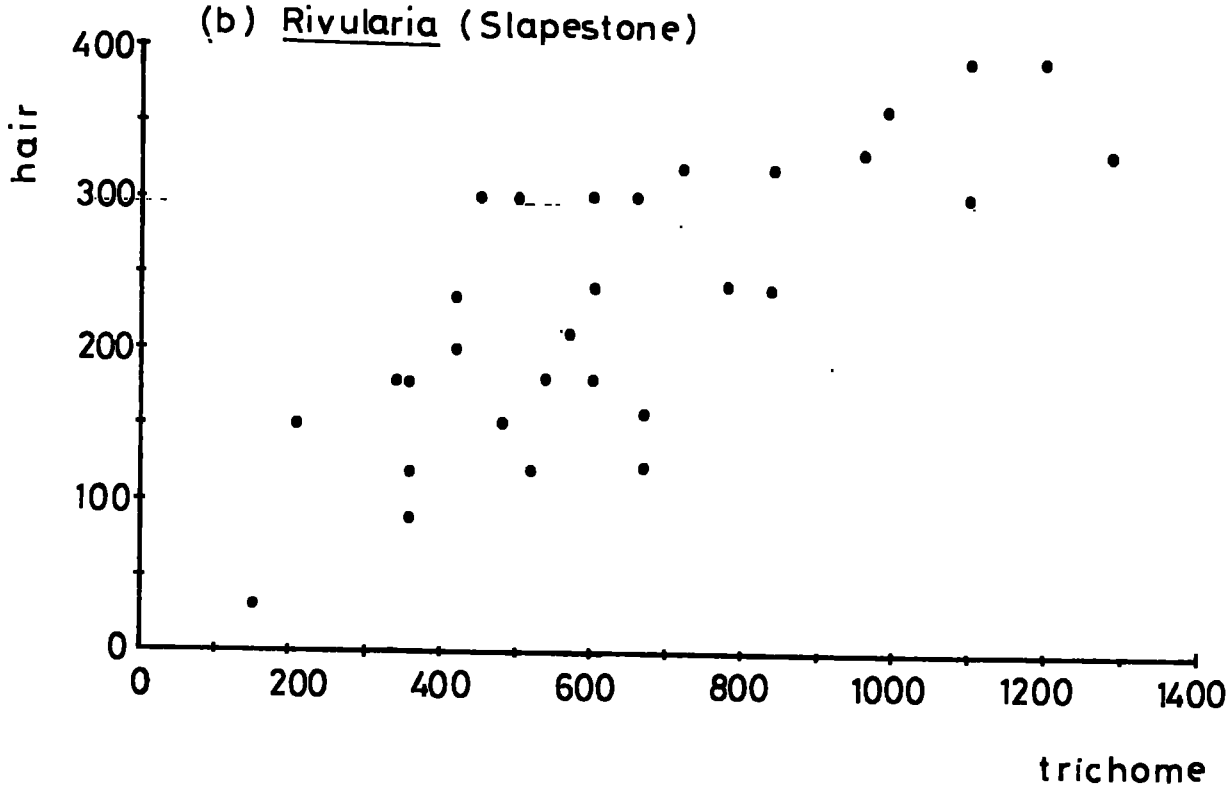
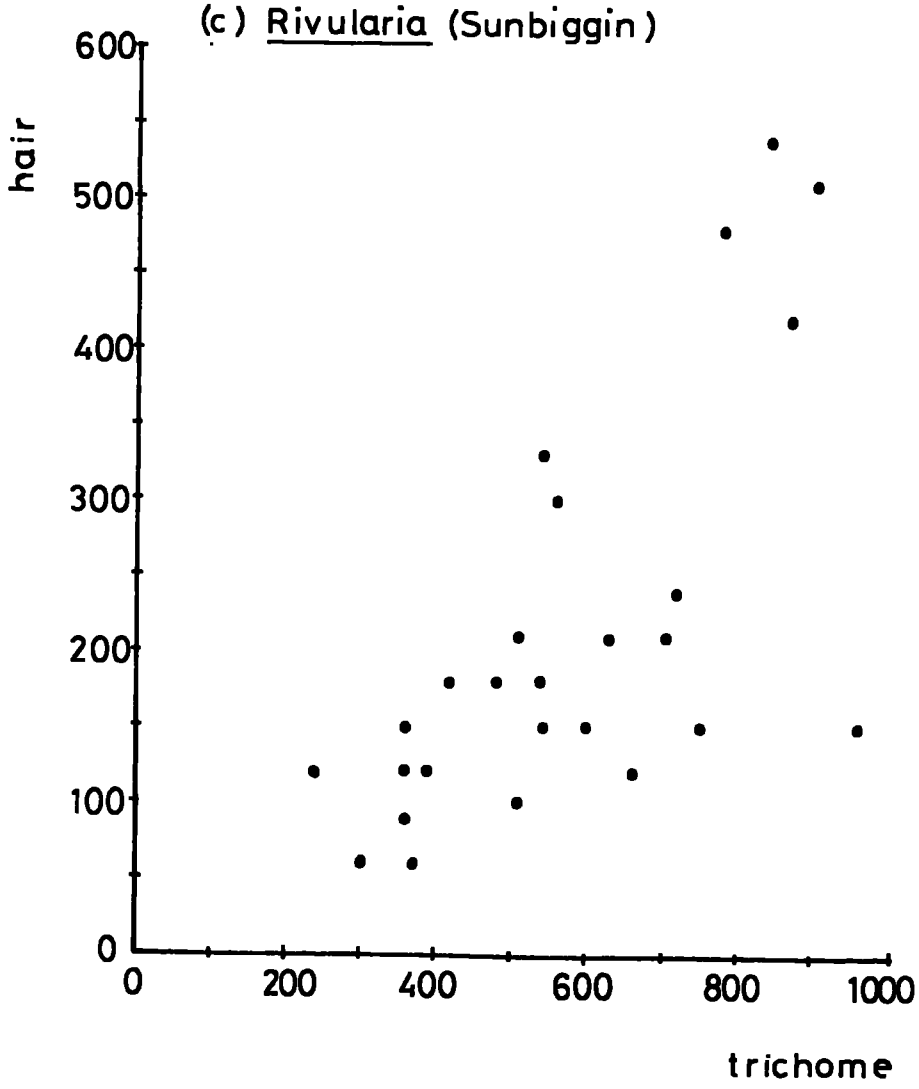


Fig. 3.9 a-c Variation in hair length and trichome length (lengths measured in μm)

(b) Rivularia (Slapestone)



(c) Rivularia (Sunbiggin)



culture, however, hairs were observed on one occasion only, in C. scopulorum (Table 3.8). This character was present when the culture was received from the Culture Collection of Algae and Protozoa, Cambridge, but was lost after several subcultures. Hairs re-appeared only once, when a bacterised culture was grown in V_{37} medium. After 21 days growth at 5‰ salinity, the material developed hairs. Both floating and attached trichomes had fairly thick sheaths and long colourless hairs (Fig. 3.10a and b). The sheaths were either colourless or dark brown in the basal region. At 20‰ salinity, the attached material possessed hairs and a brown sheath, while the floating trichomes had a colourless sheath and very few possessed hairs (Fig. 3.10c and d respectively). Very little growth occurred in the material grown at 50‰ and no hairs were observed.

A later study carried out with several other workers (Whitton, Kirkby, Peat and Sinclair 1973) resulted in the observation of hairs in 3 out of 30 strains of Rivulariaceae, cultured in AD - N medium. 27 strains of Calothrix did not form hairs, 1 strain, Calothrix sp. D. 251 possessed short hairs on some filaments and the 2 Gloeotrichia strains possessed hairs (pers. com. C. Sinclair). This work is discussed further in Section 7.6.

Field samples of Rivularia and Gloeotrichia were difficult to culture and even uni-algal cultures could not be obtained. Several observations were made of hair length, under various culture conditions and these are described here as the results may be of use to future workers.

Table 3.8 Occurrence of hairs in cultures of *Calothrix* (all species observed in AD and ASM-1 ± N)

species	hair present or absent according to	
	literature (Appendix II)	laboratory observation
Calothrix brevissima	absent	absent
C. desertica	absent	absent
C. elenkinii	present	absent
C. fusca	absent	absent
C. gracilis	present	absent
C. scopulorum	present	present
C. thermalis	present	absent
C. viguieri	-	absent
Calothrix sp. D255	-	absent
Calothrix sp. D258	-	absent
Calothrix sp. D267		absent

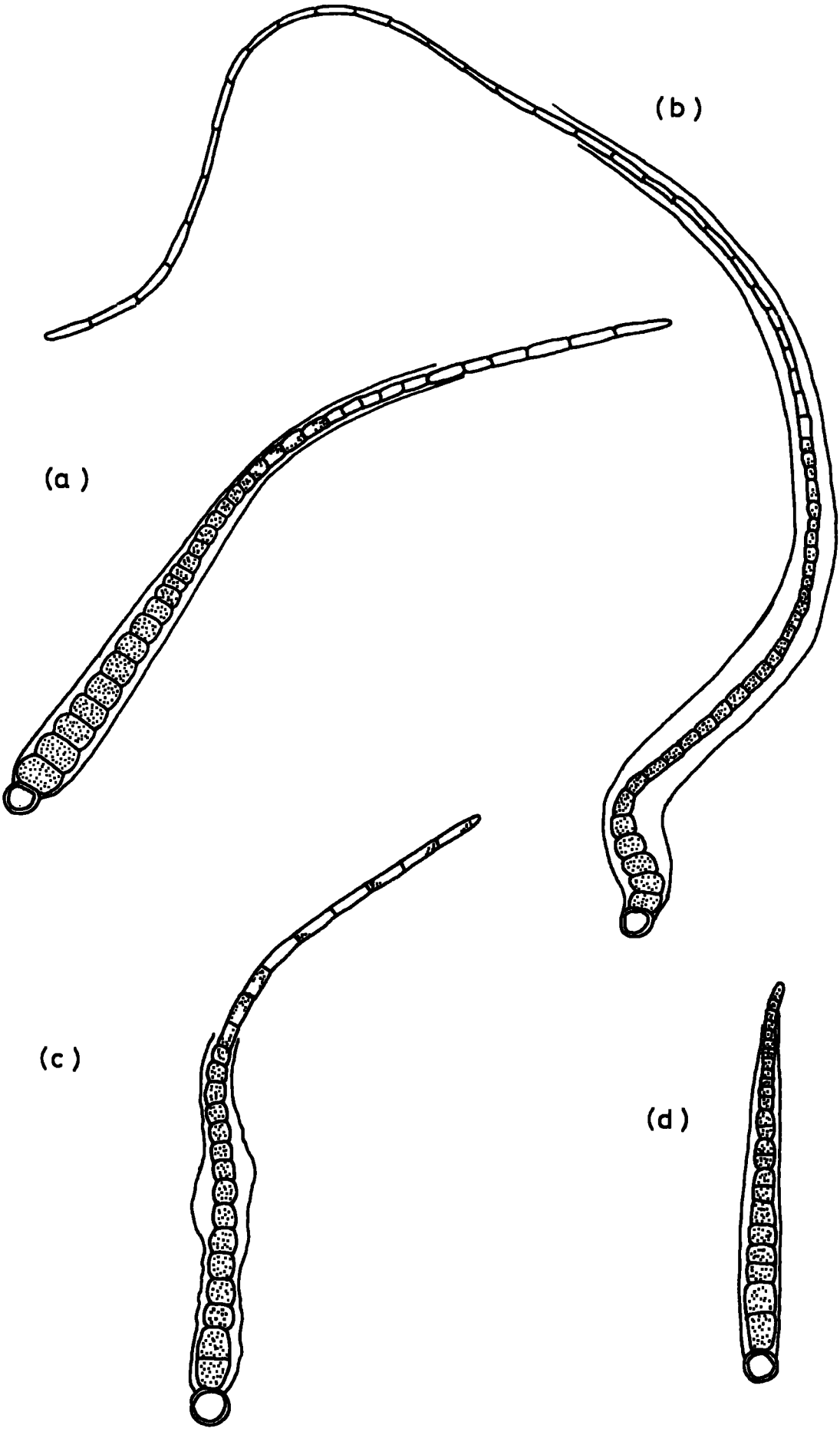


Fig.3.10 Morphology of *Calothrix scopulorum* cultured at different salinities (see p.129)

Colonies of Rivularia (Tulach) were cultured in two - N media, AD and ZD. The latter medium was a fairly dilute medium in which a uni-algal culture of R. haematites was obtained (pers. com. Zehnder). 25 ml of the 2 media were used, both containing little actidione. After being cultured for 2 months at 15°C and 1500 lux, the colonies in ZD were healther. In AD the trichomes were broken up and the cells contained many highly refractive granules. In ZD the trichomes were tapered, but there were very few hairs. If hairs were present they were very short and there was a marked constriction between the vegetative cells and the hair cells.

The effect of reducing the level of Fe from 4 mg l^{-1} to 1 mg l^{-1} was studied. In AD, at the lower levels of Fe, the trichomes contained many refractive granules, but they possessed long colourless hairs (Fig. 3.11). At the higher concentration, the material was unhealthy and hairs, if present, showed considerable variation in length. In contrast, in ZD, the hairs were short (Fig. 3.11). The longest hairs formed in ZD were formed at the higher levels of Fe.

A second set of results related to the hairs of Rivularia was obtained while attempting to grow Rivularia in culture. About 20 attempts were made and although it was possible to keep Rivularia alive for several months, the attempts on the whole were not successful; and it was not possible to culture the alga in a way suitable for experimental purposes. On one occasion, colonies were cultured at initial concentrations of inorganic phosphate of between 1.55-31.0 mg l^{-1} P (25-500 μM K_2PO_4). The results obtained

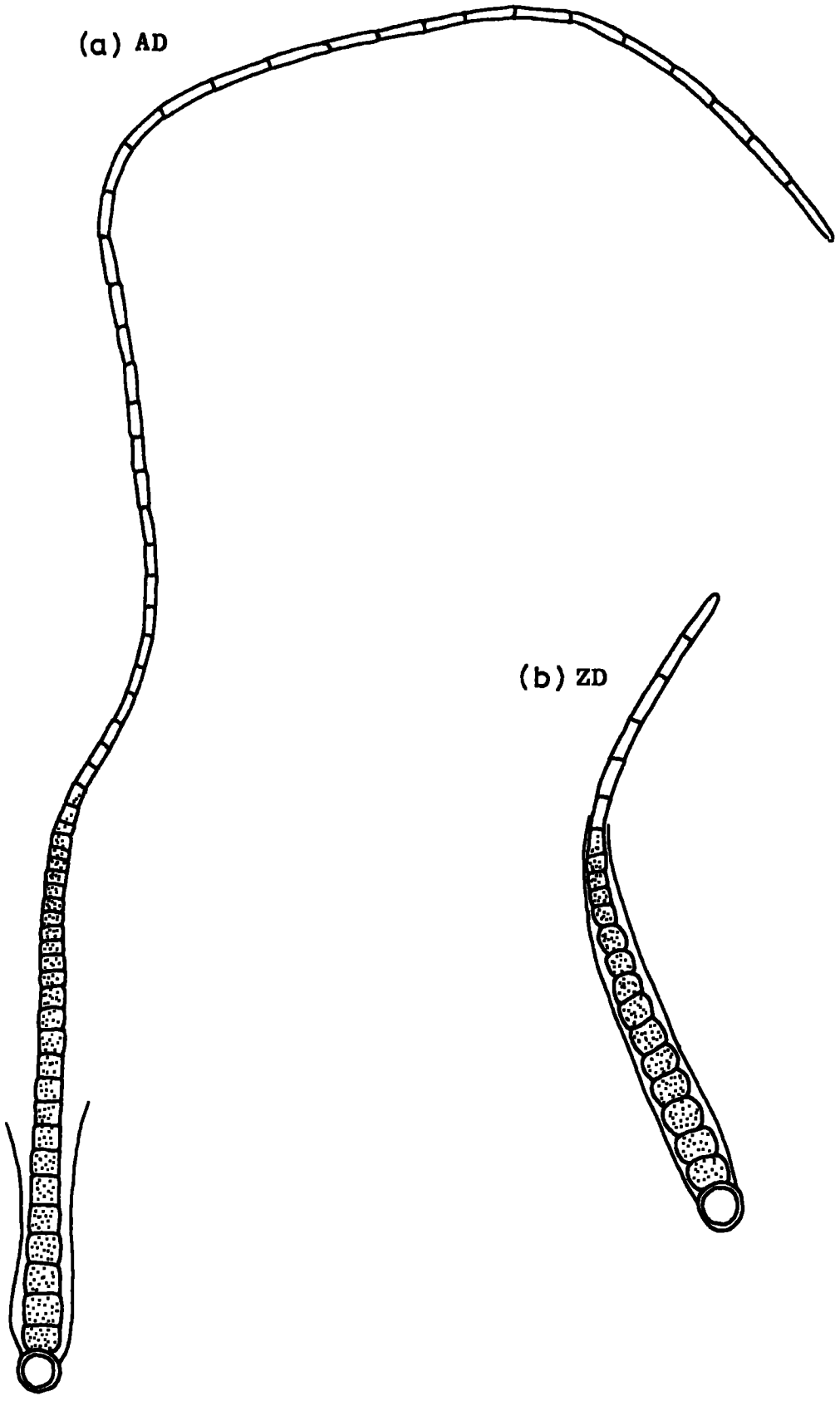


Fig. 3.11 Morphology of Rivularia (Tulach) cultured in AD and ZD media

(Fig. 3.12) indicate that at lower levels of PO_4, a greater percentage of the trichome was hair. Rivularia from Malham, used for these experiments and cultured at 3.1 and 15.5 mg l^{-1} P, produced new colonies. These were attached to the bottom of the culture flask and grew to 1-2 mm diameter.

Fogg (1969) reported that Gloeotrichia could possibly use organic phosphates and that even low concentrations of inorganic phosphate were inhibitory. The effect of organic phosphate (sodium-β-glycerophosphate) on the growth of the two Rivularia samples (from Malham and Slapestone) and Calothrix brevissima was studied. All three cultures were unhealthy in media with organic phosphate and healthy in inorganic phosphate. The influence of the initial inorganic phosphate concentration on C. brevissima is shown in Fig. 3.13. Hairs did not develop at the lower levels of inorganic phosphate.

3.5 Discussion

It is clear from microscopic examination of Rivulariaceae that there is no simple description of tapering. It is evident from Fig. 3.6 that some trichomes have a gradual taper while others show a rapid change in diameter over a small portion of the trichome. Recent work (Whitton, Kirkby, Peat and Sinclair 1973) has suggested that there may be a link between trichome shape in - N medium and the appearance of the trichomes in + N medium. These findings are discussed further in Section 7.6.

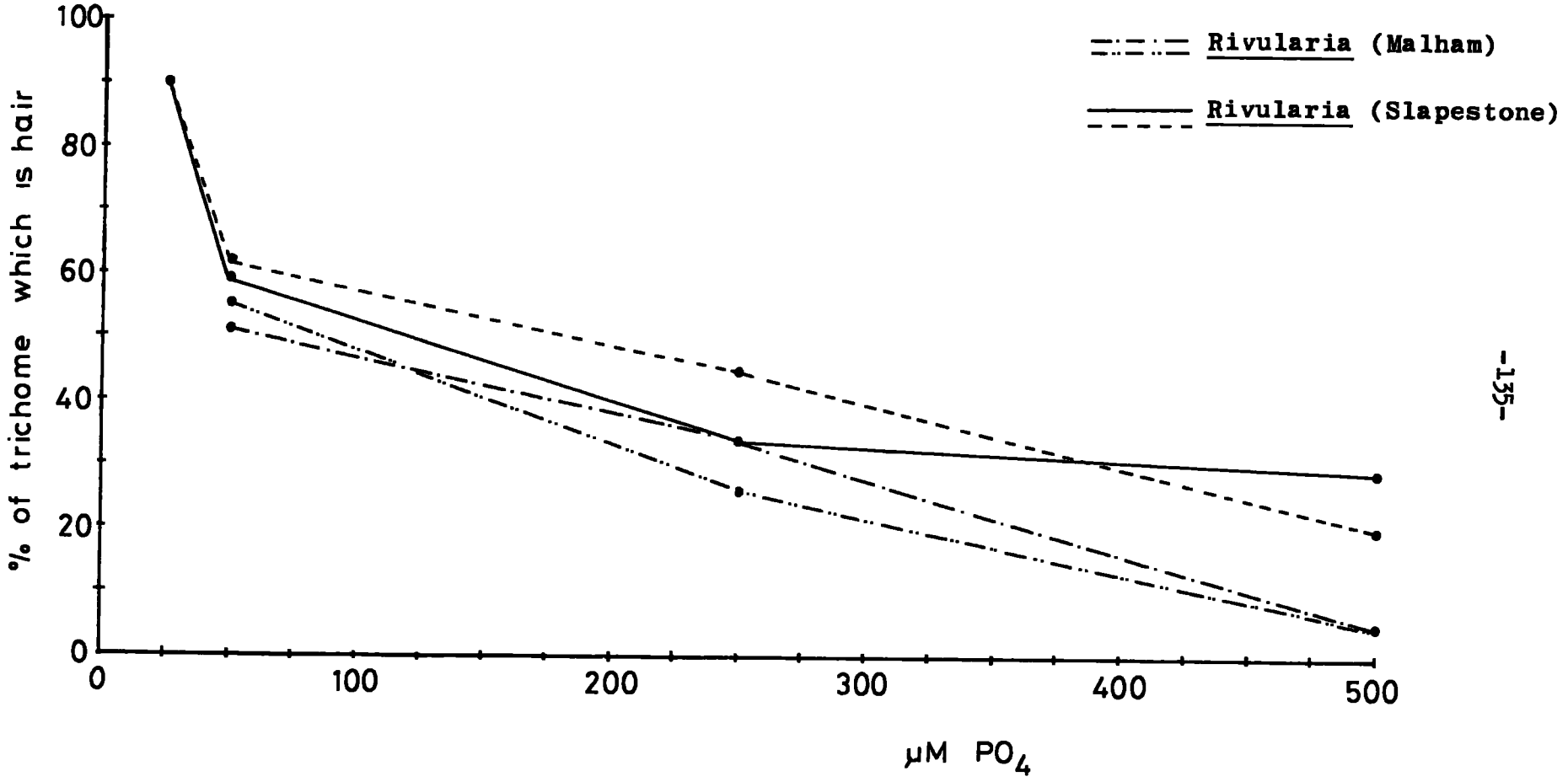


Fig. 3.12 Influence of PO_4 concentration on the hair length of Rivularia

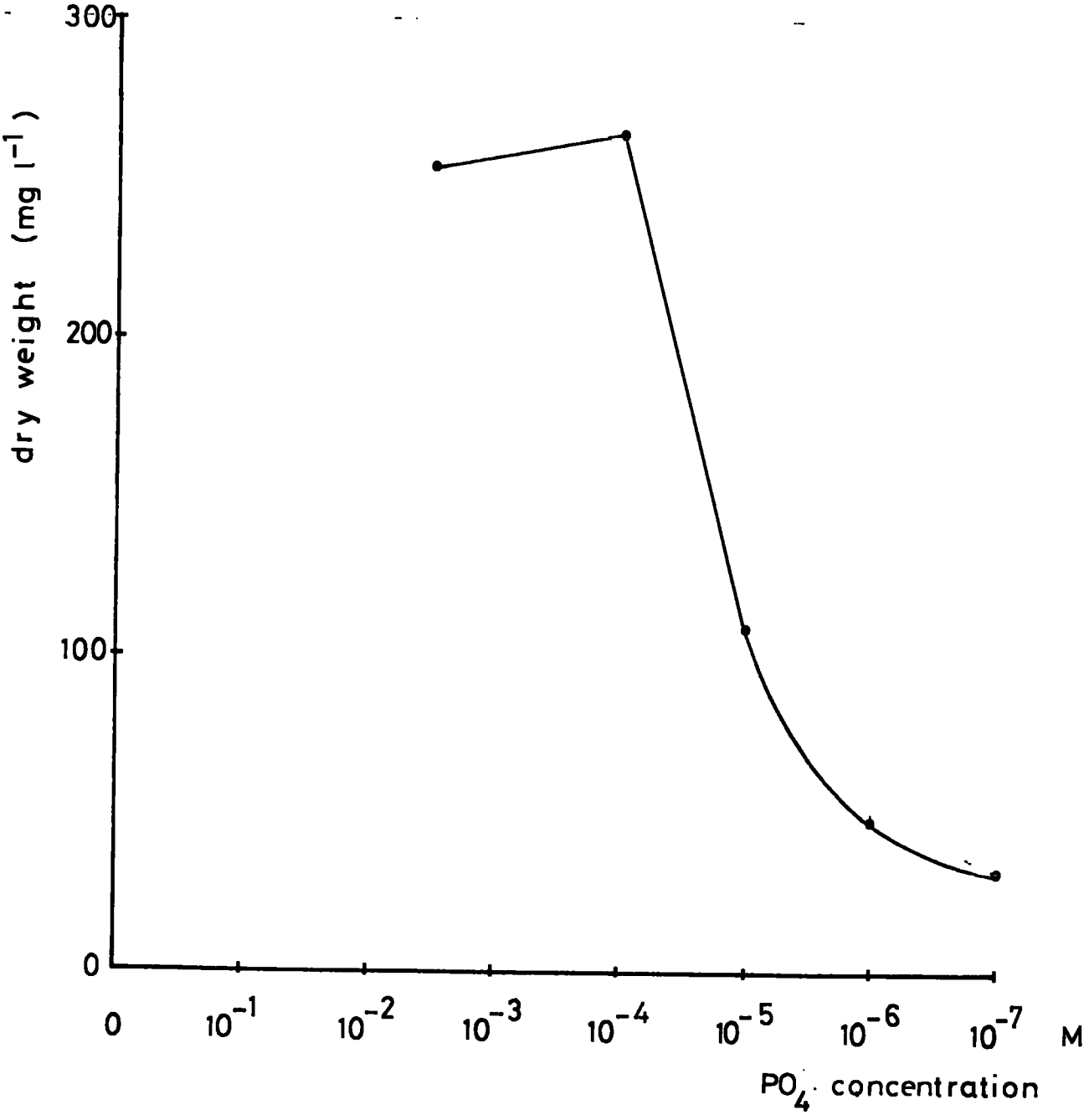


Fig. 3.13 Growth of *Calothrix brevissima* in inorganic PO_4

In spite of the difficulties described above, it was useful to be able to compare trichomes in fairly simple quantitative terms. Four indices of tapering have been devised (Section 3.2), a discussion of the use of the various indices being given in Section 7.2.

Observations of hair cells are in agreement with previous findings (Section 1.32). The cells appear colourless and are relatively narrow and elongated. The limited evidence available concerning factors which influence hair development (Section 3.4) suggests that low levels of certain nutrients tend to promote hair formation.

4 PHYSIOLOGICAL ASPECTS OF TAPERING

4.1 Introduction

As described in Section 1.36, there have been relatively few investigations related to the physiological aspects of tapering. Fay, Stewart, Walsby and Fogg (1968) reported that in the presence of NH_4-N, trichomes of Calothrix and Gloeotrichia lost their taper. The present studies are concerned with the effect of combined nitrogen on the growth and morphology of several strains of Calothrix, in particular the taper.

4.2 The effect of NH_4-N on the growth and morphology of two strains of Calothrix

4.21 Calothrix brevissima

The effect of the level of NH_4-N in the growth medium, on the growth, tapering frequency and frequency of trichomes with heterocyst is shown in Fig. 4.1. The results indicate a gradual decrease in dry weight and a more marked decrease in tapering and heterocyst frequencies, when high initial levels of NH_4-N were present in the growth medium.

The trichomes were examined with the light microscope and those growing at the lower levels of NH_4-N (0-60 mg l^{-1} NH_4Cl, 0-14.5 mg l^{-1}N) were healthy in appearance and blue-green in colour. At the two highest levels of NH_4-N, the cultures appeared orange in colour when viewed macroscopically. Under the microscope, most of the trichomes were orange-yellow; however, those grown at 19.25 mg l^{-1}N

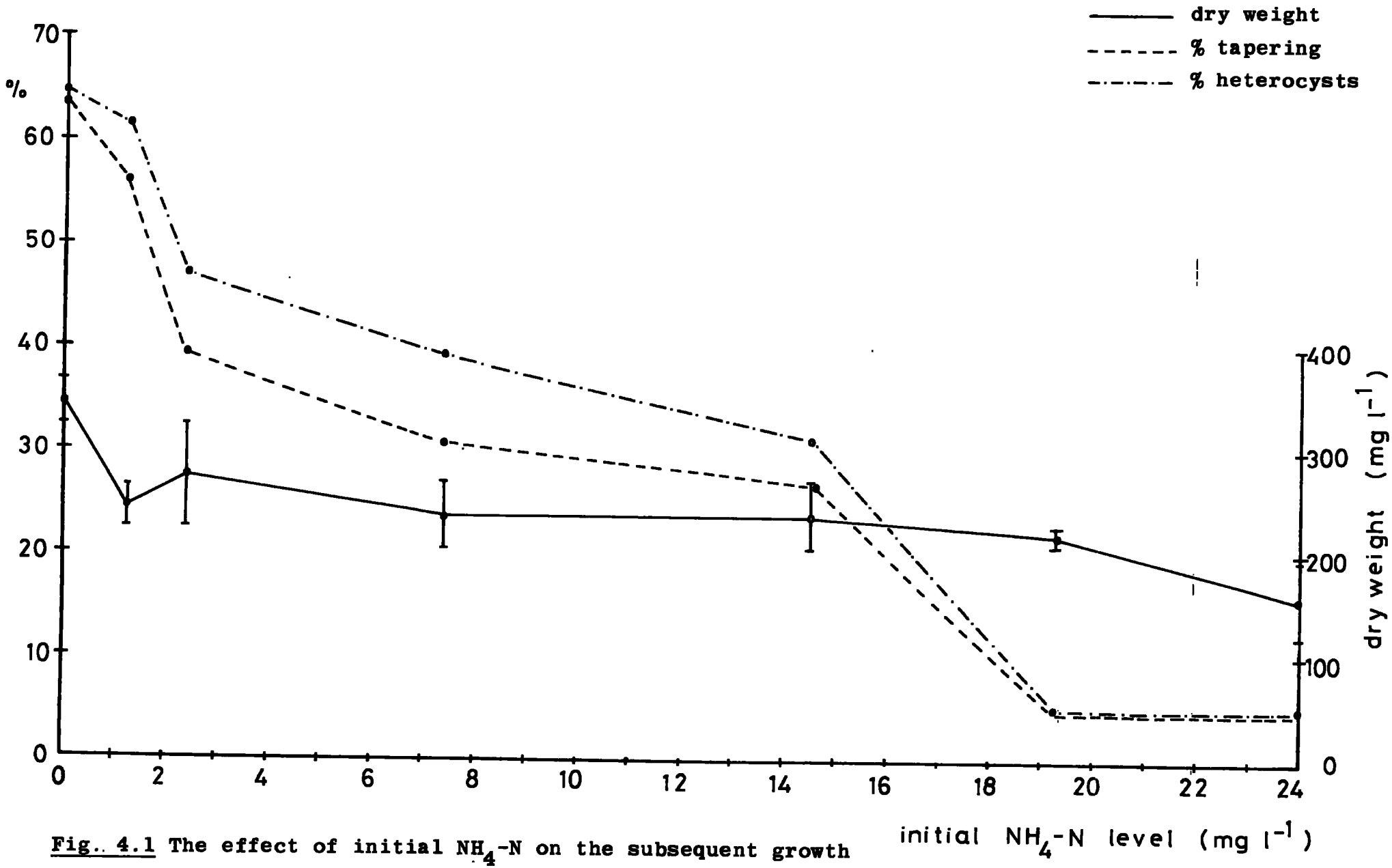


Fig. 4.1 The effect of initial NH_4-N on the subsequent growth and morphology of Calothrix brevissima

and in the middle of a clump of trichomes, were still blue-green. At the two highest levels of NH_4-N, there was a decrease in the percentage of trichomes which tapered and which possessed heterocysts. These marked changes (Fig. 4.2) are probably due partly to the cultures being unhealthy at the higher levels of NH_4-N.

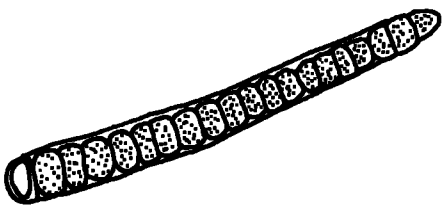
The results were checked by subculturing C. brevissima for a second time, to the same concentration of NH_4-N (e.g. 1.25 mg l^{-1}N to 1.25 mg l^{-1}N). Total growth was greater after the second subculture at all levels of NH_4-N, except the highest (Fig. 4.3). There was a gradual decrease in the frequency of tapering and the frequency of trichomes with heterocysts, with increasing NH_4-N concentration. These results were similar to those observed during the first subculture.

Algal material from each NH_4-N concentration was also subcultured to - N medium, in order to discover whether or not the percentage of tapered trichomes and the percentage of trichomes with heterocysts would increase again. The results, given in Fig. 4.4, show that in most cases there was a considerable increase when the alga was subcultured to - N medium.

4.22 Calothrix viguieri

A similar set of experiments was carried out using C. viguieri. The results of dry weight determinations, when the alga was grown at different initial levels of NH_4-N, are shown in Table 4.1. C. viguieri grew well at the lower concentrations of NH_4-N, but

(a) Calothrix brevissima in - N medium



(b) Calothrix brevissima in + N medium

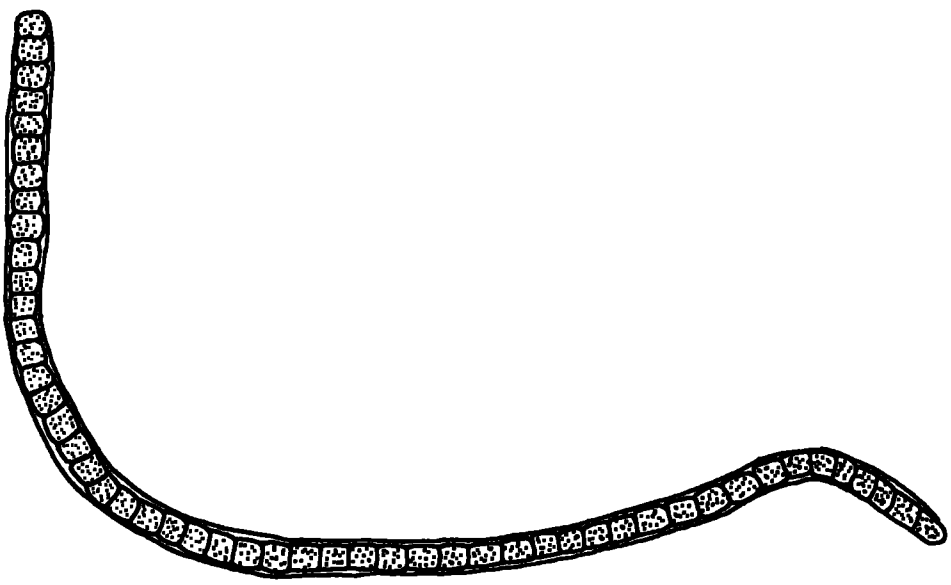


Fig. 4.2 Morphology of Calothrix brevissima in + NH_4-N media and NH_4-N

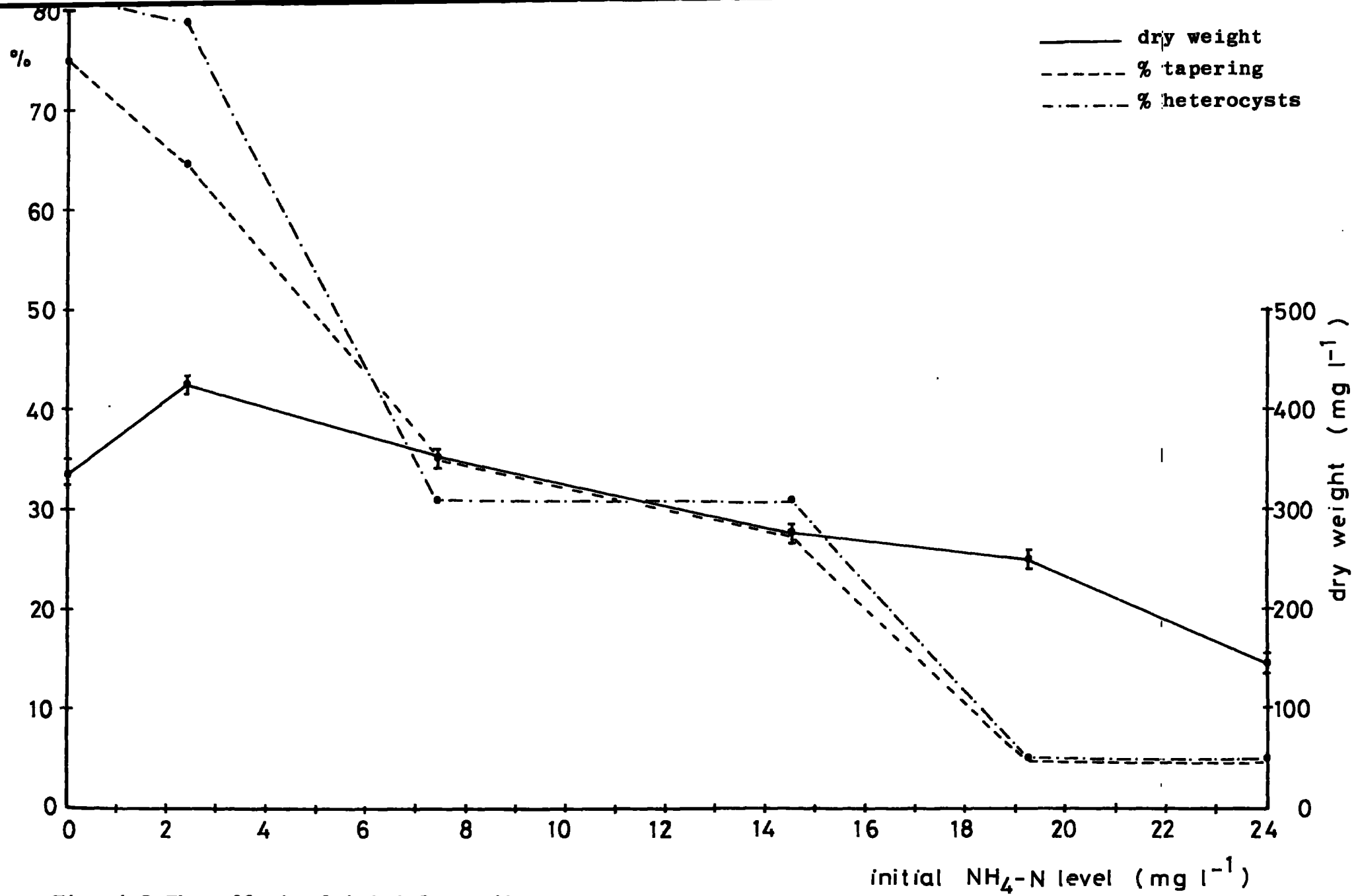


Fig. 4.3 The effect of initial NH_4-N on subsequent growth and morphology of Calothrix brevissima (second subculture)

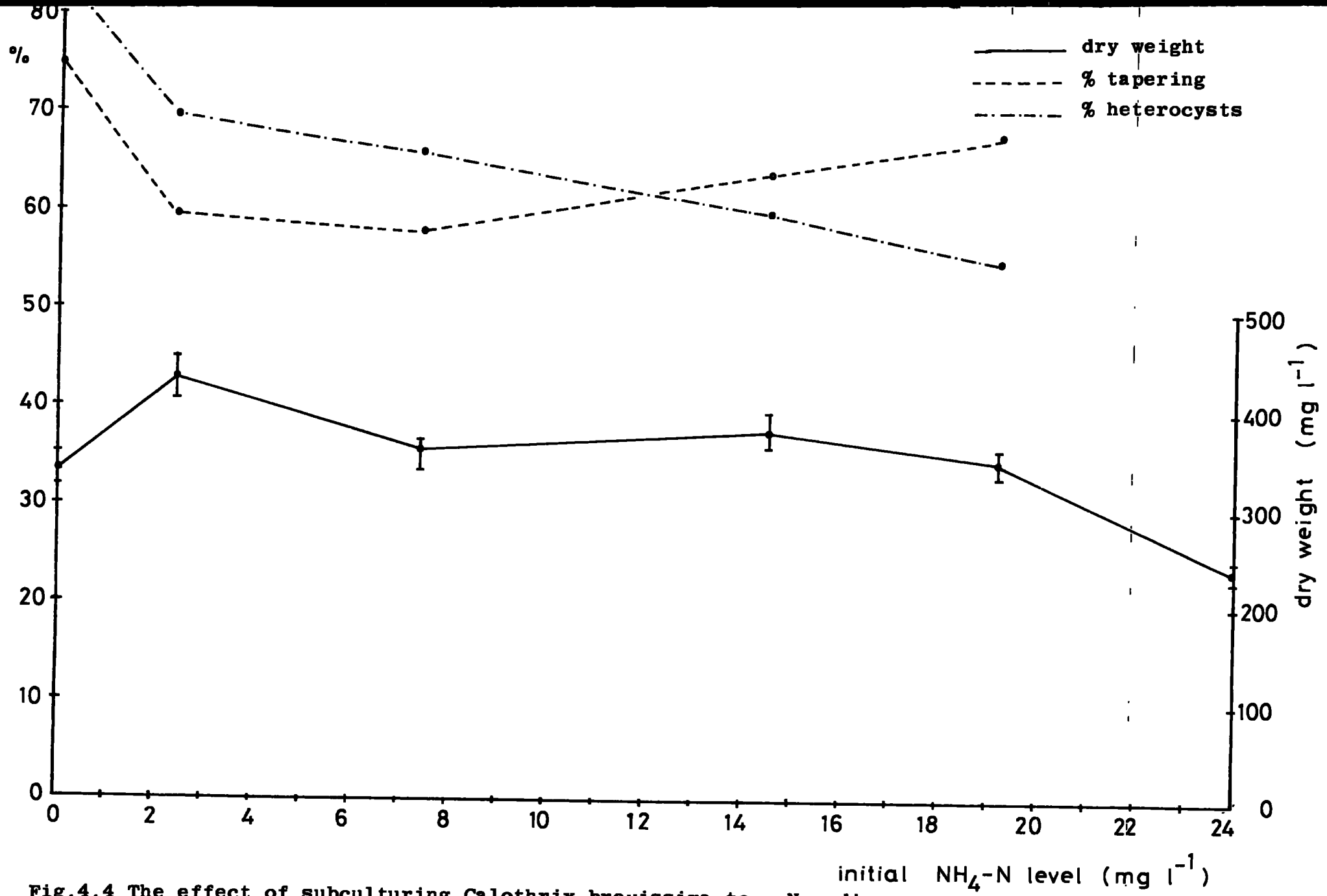


Fig.4.4 The effect of subculturing *Calothrix brevissima* to - N medium

Table 4.1 The effect of initial NH_4-N on the subsequent growth and morphology of Calothrix viguieri; semiquantitative estimates used in assessing tapering and heterocyst frequencies.

initial NH_4-N(mg l^{-1})	dry weight (mg l^{-1})	tapering frequency	heterocyst frequency
0	299 $\pm$ 12.3	> 80%	> 80%
3.5	550 $\pm$ 14.9	> 80%	> 80%
7.0	536 $\pm$ 3.6	> 80%	> 80%
14.5	185 $\pm$ 9.4	60-80%	60-80%
29.2	(Unhealthy)	10-30%	10-30%

showed only slight growth at concentrations above 14.5 mg l^{-1} NH_4-N. Tapering and heterocyst frequencies were recorded in a semi-quantitative way (Table 4.1). The results show a gradual decrease in the frequencies of heterocysts and tapering, with increases in the initial concentration of NH_4-N. Typical filaments of C. viguieri cultured at different levels of NH_4-N are illustrated in Fig. 4.5. The diagrams show differences in the 'tapered appearance' and also differences in the cell shape in various parts of the trichomes.

It is obvious that tapering has been lost at the highest level of NH_4-N, but it is difficult to compare 'tapering' at the other levels. Furthermore, at 14.5 mg l^{-1} NH_4-N, the trichomes were developing a different morphology, characteristic of Hammatoidea. The need for a semi-quantitative estimate of tapering is evident from Fig. 4.5.

An indication of nitrogen-fixing ability of C. viguieri was obtained by means of the acetylene reduction assay technique. A comparison of the mean acetylene reduction results, of cultures grown at different initial levels of NH_4-N is given in Fig. 4.6. There was a decrease in acetylene reduction with an increase in the initial level of NH_4-N in the growth medium. This decrease was very marked at the highest level of 14.5 mg l^{-1} NH_4-N, although there was still a relatively high percentage of trichomes with heterocysts.

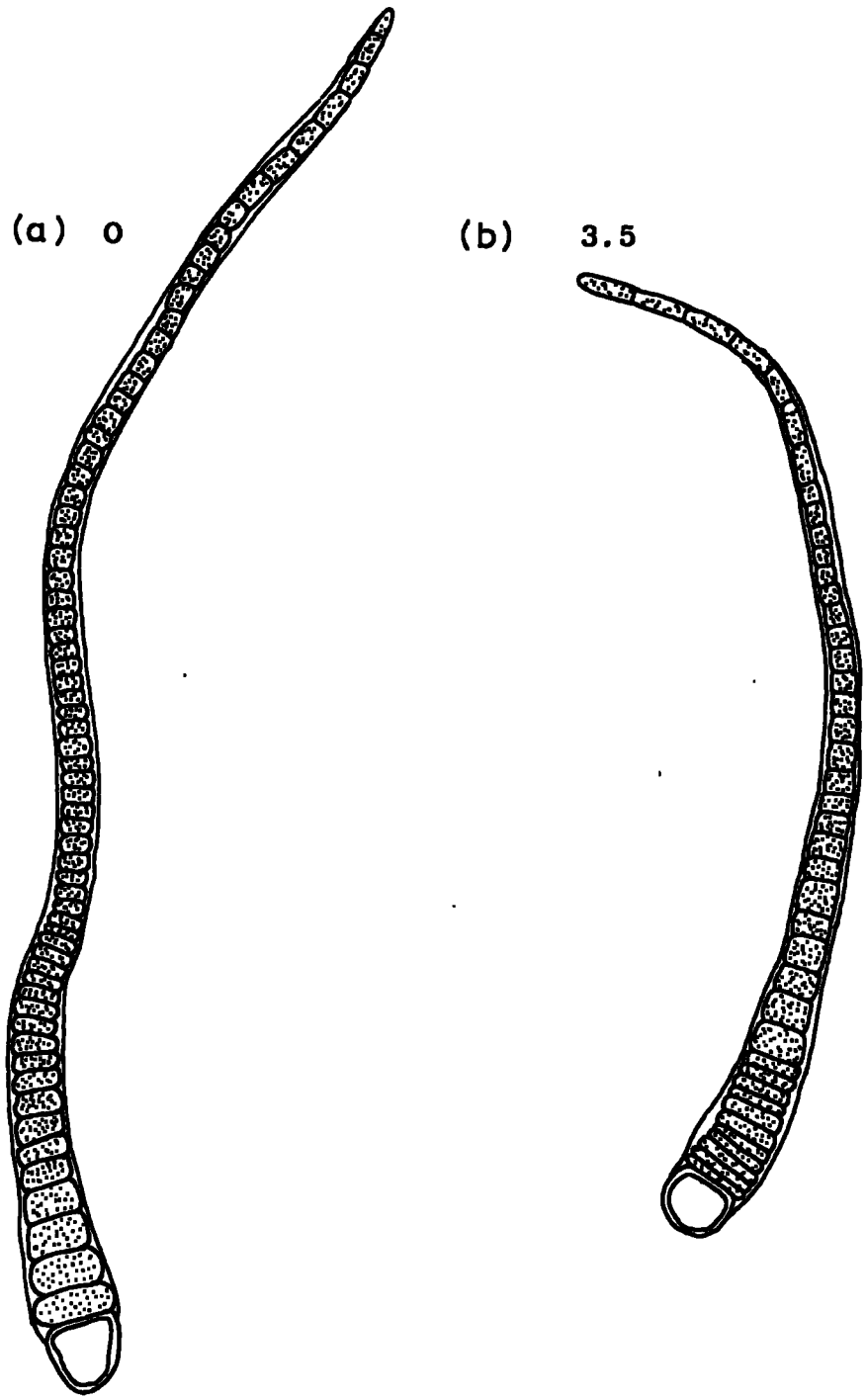
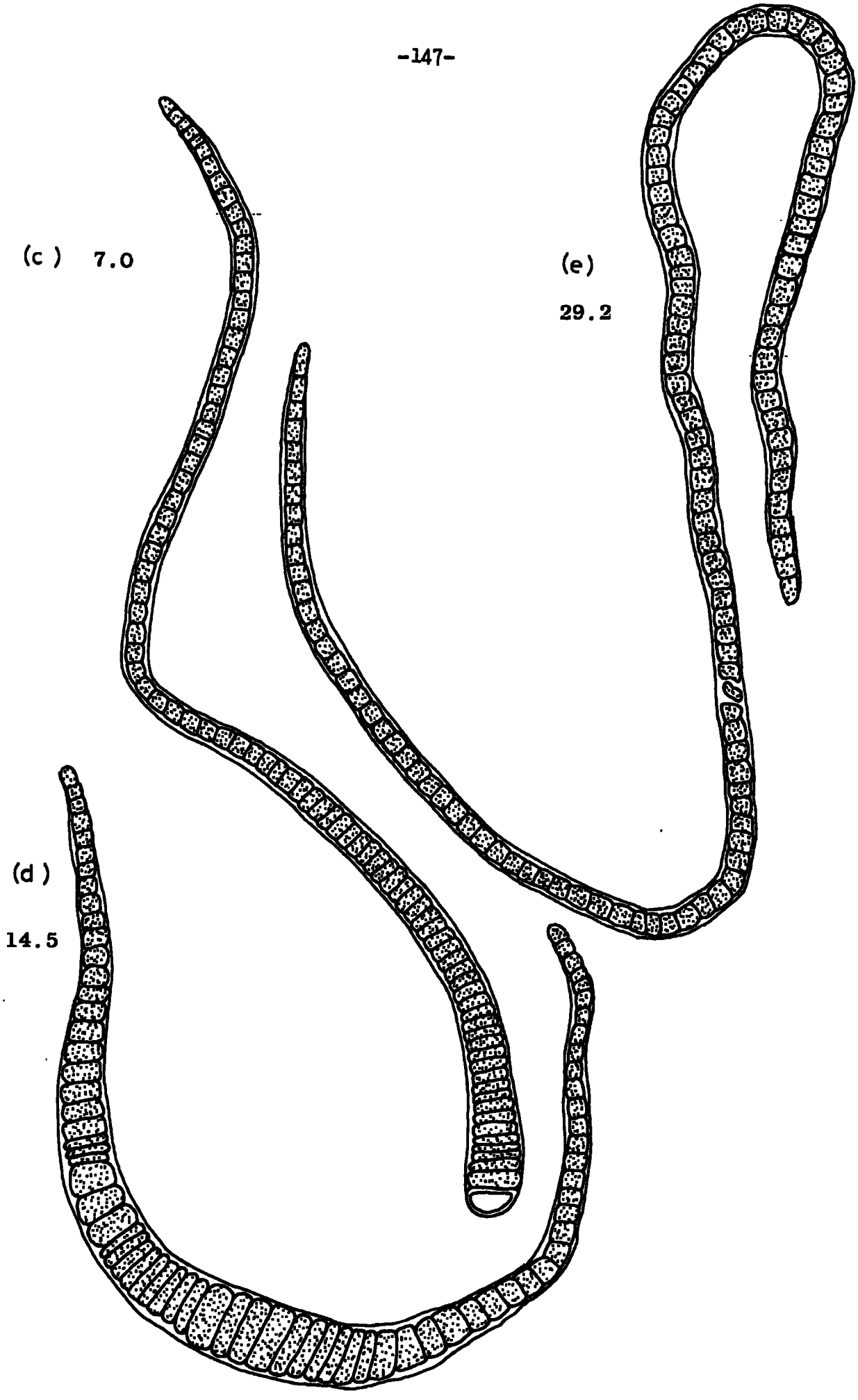


Fig. 4.5a-e Morphology of Calothrix viguieri cultured at different initial levels of NH_4-N (concentrations in mg l^{-1})


(c) 7.0
(e)
29.2
(d)
14.5

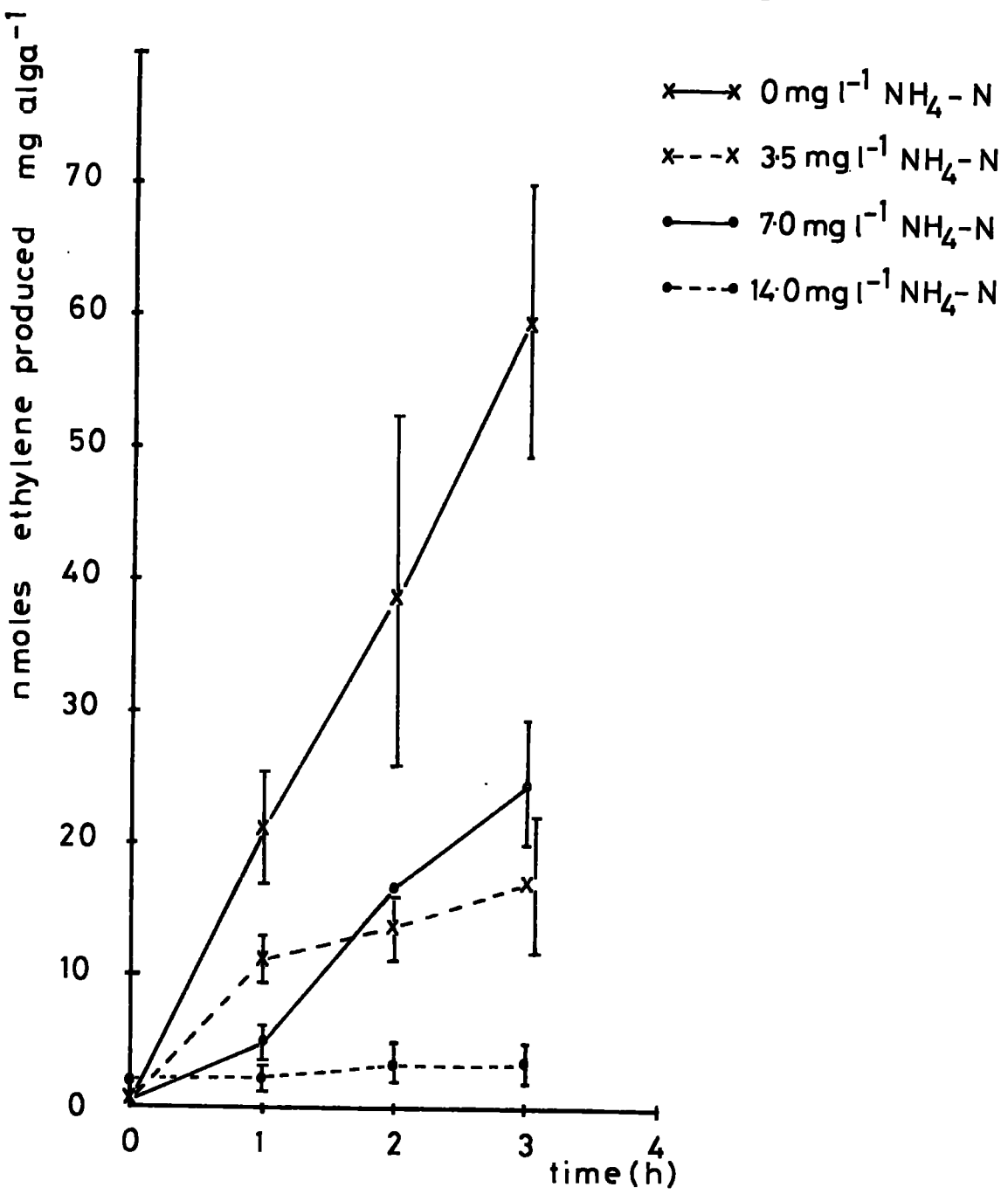


Fig. 4.6 The effect of initial NH_4-N on acetylene reduction of Calothrix viguieri

4.3 The effect of NO_3-N on the growth and morphology of two strains of Calothrix

This series of experiments, using NO_3-N, were carried out in order to study changes in tapering and nitrogen-fixing ability in more detail. The two strains used for these experiments were C. viguieri and C. scopulorum. (The latter strain was used in place of C. brevissima, which had unfortunately become contaminated and subsequently proved difficult to re-obtain in an axenic state).

4.31 Calothrix viguieri

C. viguieri was cultured for 32 days in + N and - N media. During this time, changes in dry weight, tapering frequency and heterocyst frequency were recorded. The results are shown in Fig. 4.7 and 4.8. The increase in dry weight was greater in + N medium. The percentage of trichomes which tapered and the percentage which possessed heterocysts remained fairly constant in the - N medium, while in the + N medium, there was a marked decrease in both tapering and heterocysts frequencies. The decrease in heterocyst frequency was very noticeable at an early stage of growth, compared to the decrease in tapering frequency, which occurred gradually. The frequency of heterocysts was reduced by 90% compared with the frequency of tapering which was reduced by 40%.

Changes in the morphology of C. viguieri are shown in Fig. 4.9. The cultures in the + N and - N media showed a similar 'tapered appearance' up to day 12, but this time there were marked differences in the shapes of the cells. After 23 days in + N medium, the trichomes became Hammatoidea-like and tapered at both ends. The trichomes in the + N medium for 32 days, did not become parallel.

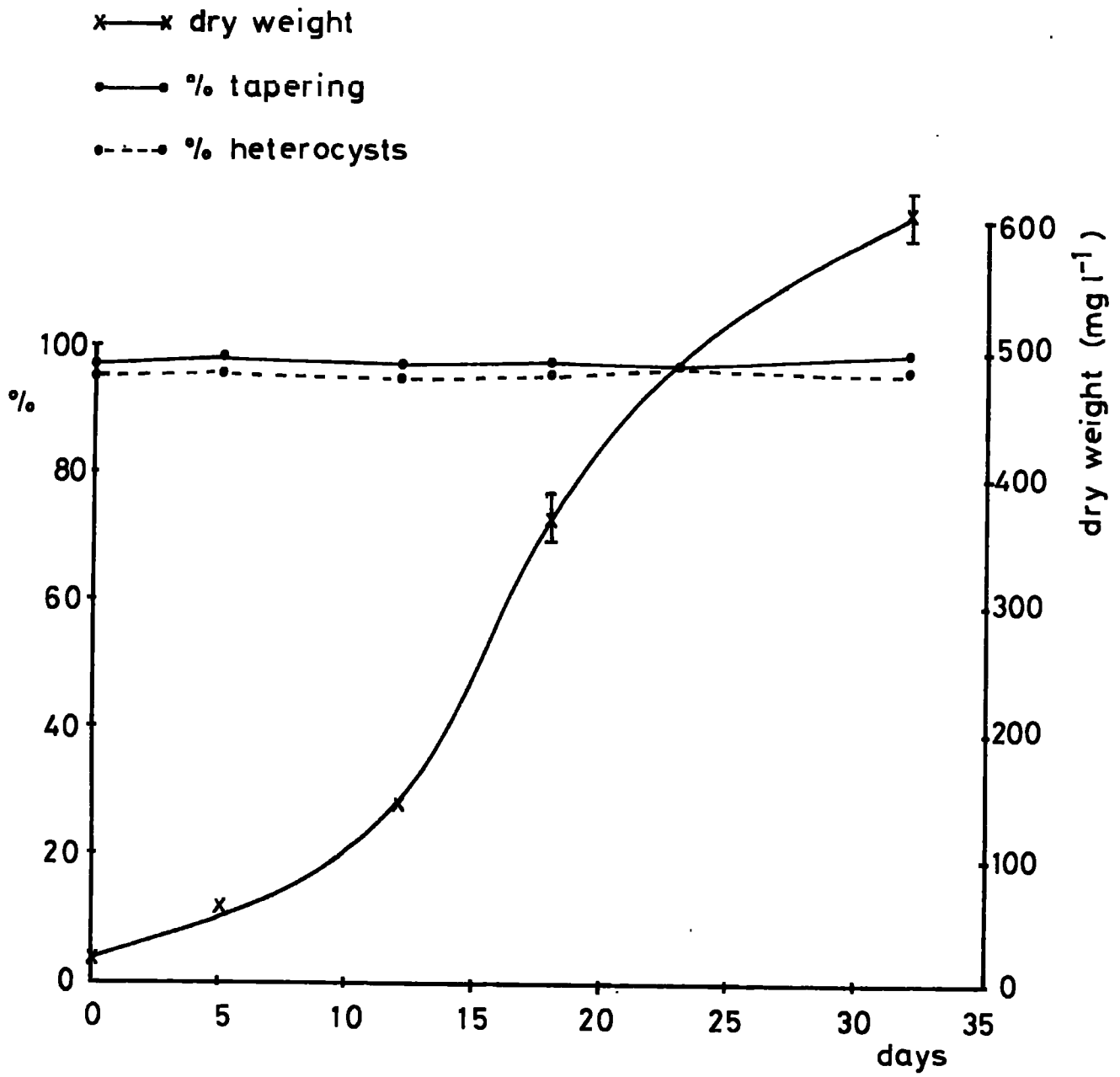


Fig. 4.7 Growth and morphology of Calothrix viguieri in - N medium

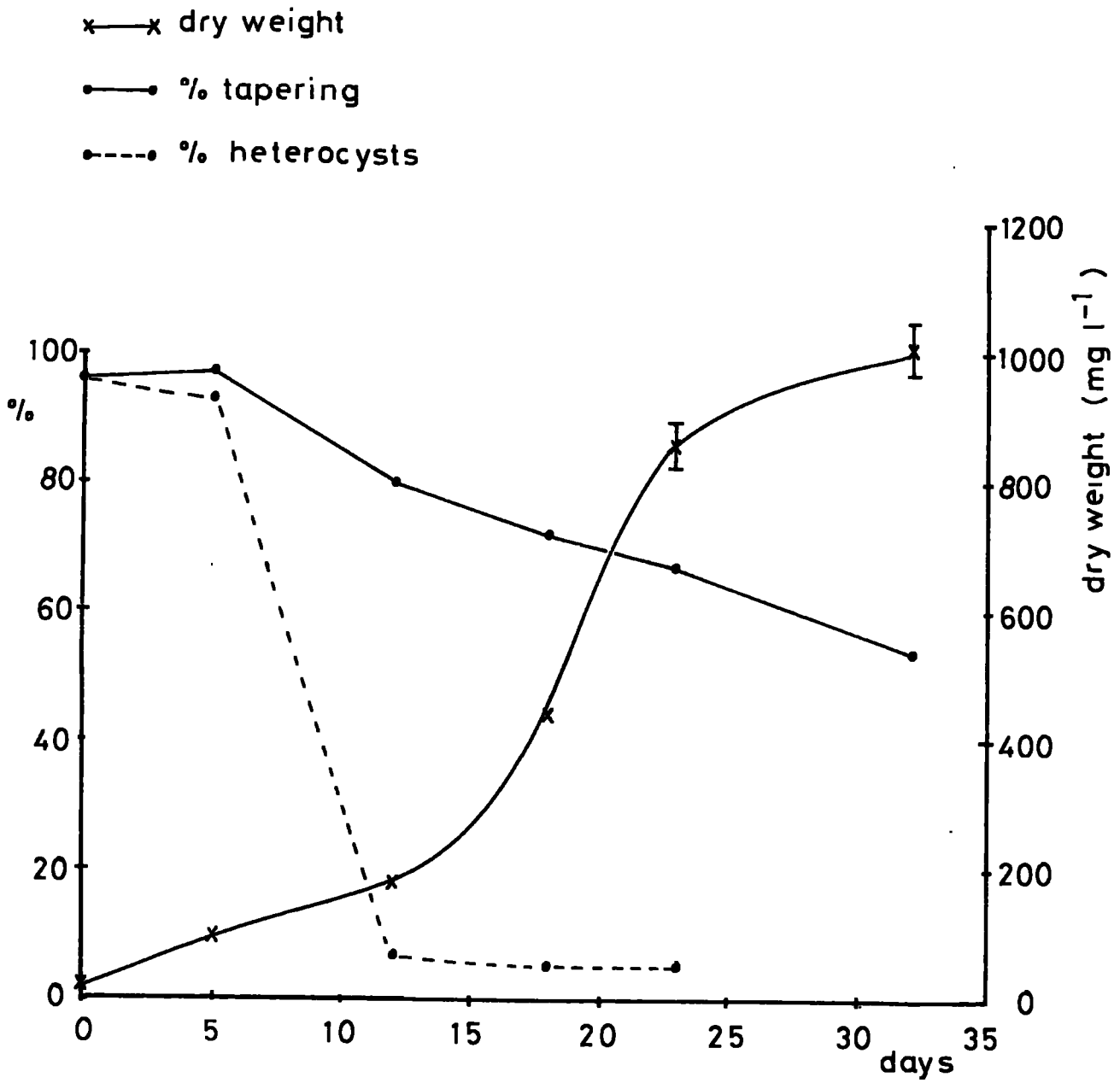


Fig. 4.8 Growth and morphology of *Calothrix viguieri* in + N medium

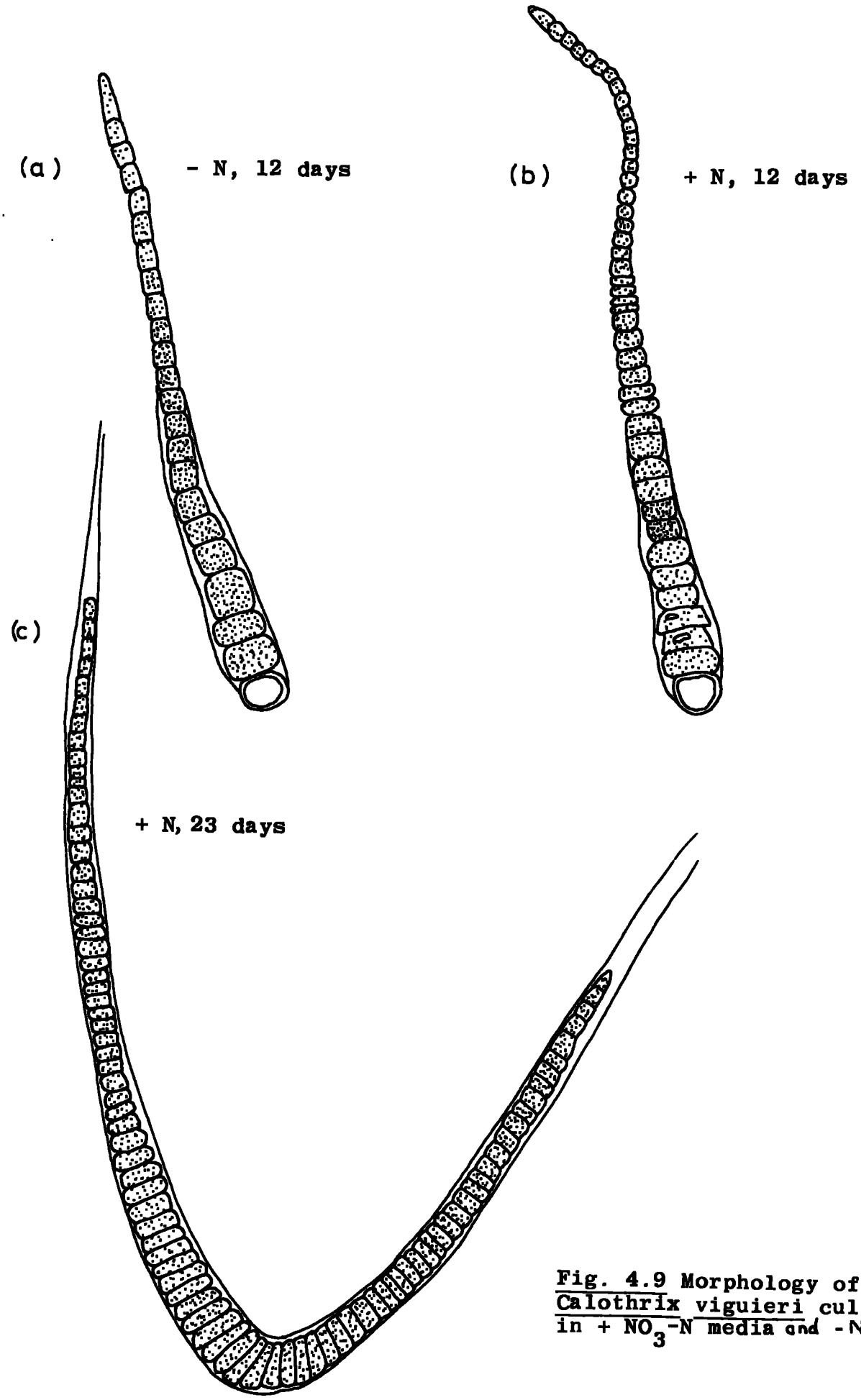


Fig. 4.9 Morphology of Calothrix viguieri cultured in + NO_3-N media and - NO_3-N

The mean basal and apical widths of the trichomes cultured in the + N and - N medium are given in Table 4.2. The changes in tapering of the trichomes were measured in terms of the four indices of tapering described in Section 3.21, the results being given in Table 4.3.

The estimates of tapering indicate that in - N media tapering remains fairly constant. In + N media there was a marked decrease in tapering on and after 18 days of growth. Material cultured in AD medium with NaCl-Na at the same level as $NaNO_3$-Na, had a similar appearance to material cultured in the - N medium.

Some indication of the nitrogen-fixing ability of C. viguieri in - N and + N media was obtained using the acetylene reduction assay. The results obtained using material cultured in - N medium are shown in Fig. 4.10 and Table 4.4. The results indicate a high rate of acetylene reduction during the early stages of growth in - N medium, followed by a gradual decrease in the rate of acetylene reduction. In + N medium, acetylene reduction was low throughout the growth period, the results being given in Table 4.5.

4.32 Calothrix scopulorum

C. scopulorum was cultured for 31 days in + N and - N media. The changes in dry weight, tapering frequency and the percentage of trichomes with heterocysts are shown in Fig. 4.11 and 4.12. In the - N medium, the percentage of trichomes which tapered and the percentage which possessed heterocysts remained at a fairly high level throughout the period of growth. In the + N medium there was

Table 4.2 Basal and apical widths of *Calothrix viguieri* cultured in ± NO_3-N media.

widths in μm.

age in days	basal width		apical width	
	- N	+ N	- N	+ N
5	9.65 ± 0.32	9.55 ± 0.34	3.62 ± 0.15	3.80 ± 1.39
12	10.85 ± 0.28	10.30 ± 0.31	4.35 ± 0.08	4.47 ± 0.20
18	8.60 ± 0.56	7.10 ± 0.28	3.58 ± 0.16	4.65 ± 0.18
23	10.45 ± 0.33	7.50 ± 0.27	3.65 ± 0.14	5.60 ± 0.18
32	10.10 ± 0.30	5.00 ± 0.19	4.40 ± 0.14	3.90 ± 0.22

Table 4.3 Change in the tapering of Calothrix viguieri cultured in + NO_3-N and - NO_3-N media. T_1, T_2, T_3 and T_4 are tapering estimates

age in days	T_1		T_2		T_3		T_4		Number of trichomes, out of 30 with no taper	
	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N
5	6.0 ± 0.3	5.8 ± 0.3	60.2 ± 1.4	59.2 ± 1.6	3.2 ± 0.2	3.2 ± 0.3	33.1 ± 3.4	35.7 ± 4.1	0	0
12	6.5 ± 0.2	5.8 ± 0.3	59.4 ± 1.0	58.3 ± 1.7	-	-	-	-	0	0
18	5.0 ± 0.6	2.5 ± 0.3	52.2 ± 3.8	31.8 ± 3.5	1.7 ± 0.3	2.9 ± 0.5	17.5 ± 2.2	35.1 ± 5.1	1	5
23	6.9 ± 0.4	1.9 ± 0.3	64.2 ± 1.7	24.1 ± 3.2	3.2 ± 0.4	2.0 ± 0.3	30.9 ± 3.5	27.5 ± 4.4	0	7
32	5.7 ± 0.3	1.1 ± 0.2	53.1 ± 1.9	21.8 ± 3.0	1.6 ± 0.1	0.8 ± 0.1	14.4 ± 1.0	16.9 ± 3.0	0	10

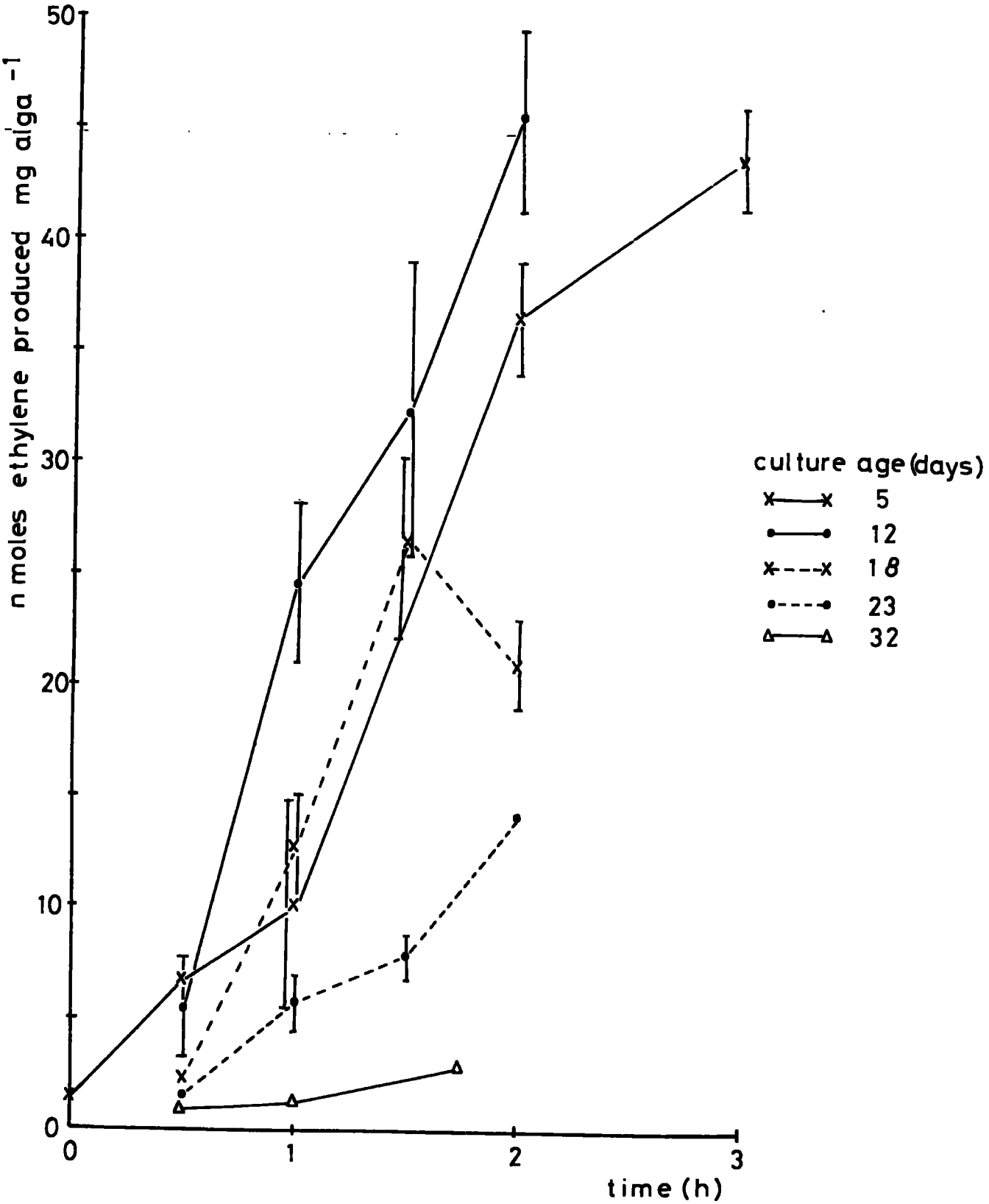


Fig 4.10 Acetylene reduction assay of Calothrix viguieri cultured in - N medium

Table 4.4 Acetylene reduction assay of Calothrix viguieri cultured in - NO_3-N medium.

total nmoles C_2H_4 produced mg $alga^{-1}$

days growth / incubation time (h)	5	12	18	23	32
0	1.44 ± 0.1	-	-	-	-
0.5	6.91 ± 0.1	5.38 ± 2.2	2.42 ± 0.4	1.70 ± 0.2	0.94 ± 0.3
1.0	10.14 ± 4.8	24.68 ± 3.6	13.16 ± 2.5	5.89 ± 1.2	1.38 ± 0.1
1.5	-	32.28 ± 6.8	26.65 ± 4.6	7.96 ± 1.0	-
1.75	-	-	-	-	32.5 ± 0.1
2.0	36.67 ± 2.6	45.50 ± 3.9	20.86 ± 1.9	14.23 ± 0.3	-
3.0	43.83 ± 2.3	-	-	-	-

Table 4.5 Acetylene reduction assay of Calothrix viguieri cultured in + NO_3-N medium.

nmoles C_2H_4 produced mg alga^{-1}

days growth	5	12	18	23	32
incubation time (h)					
0	0.45 ± 0.0	0.74 ± 0.1	1.71 ± 0.9	0.87 ± 0.0	1.15 ± 0.1
2	0.44 ± 0.0	0.68 ± 0.0	1.46 ± 0.7	0.55 ± 0.0	0.90 ± 0.0

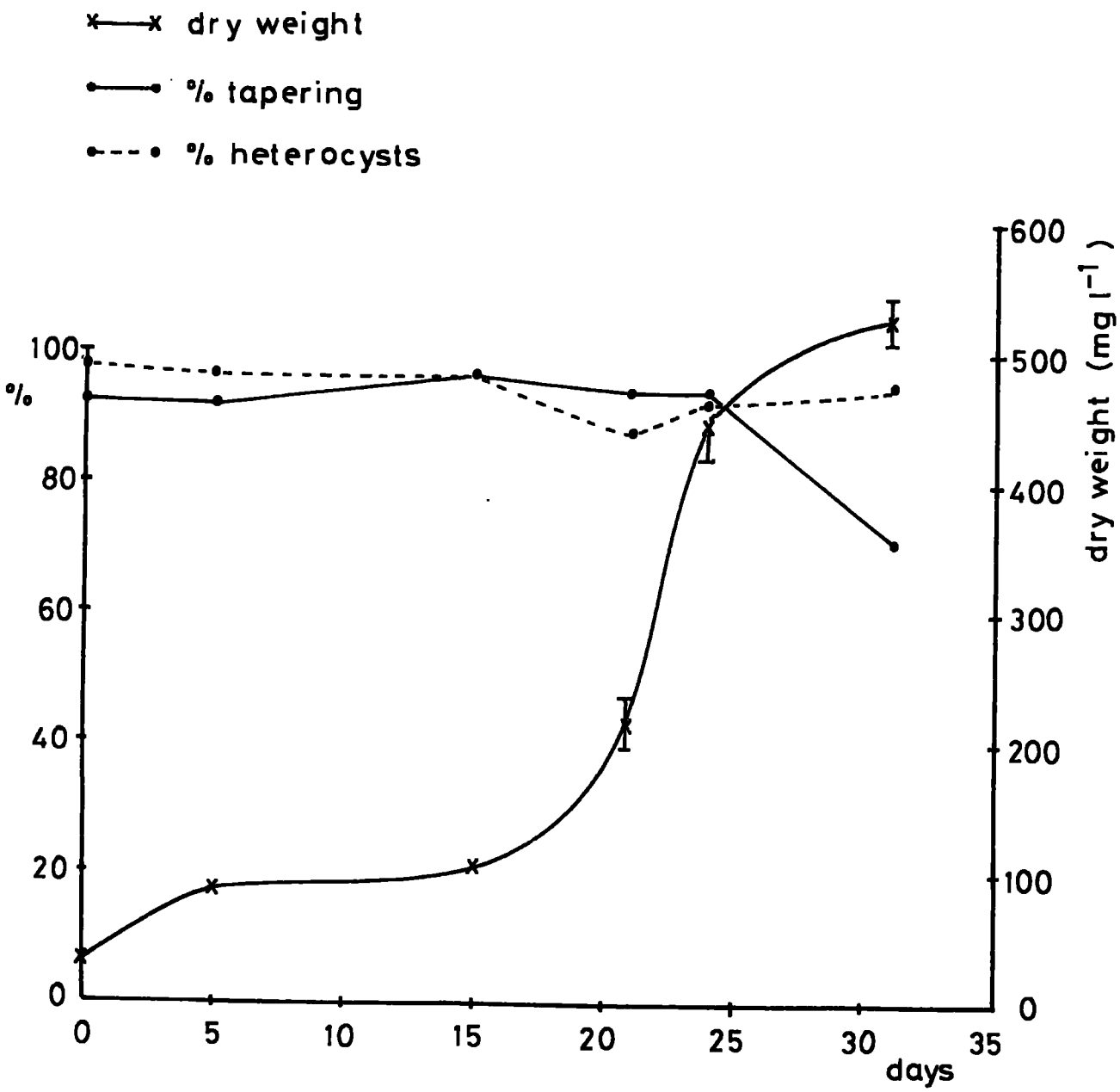


Fig. 4.11 Growth and morphology of Calothrix scopulorum in - N medium

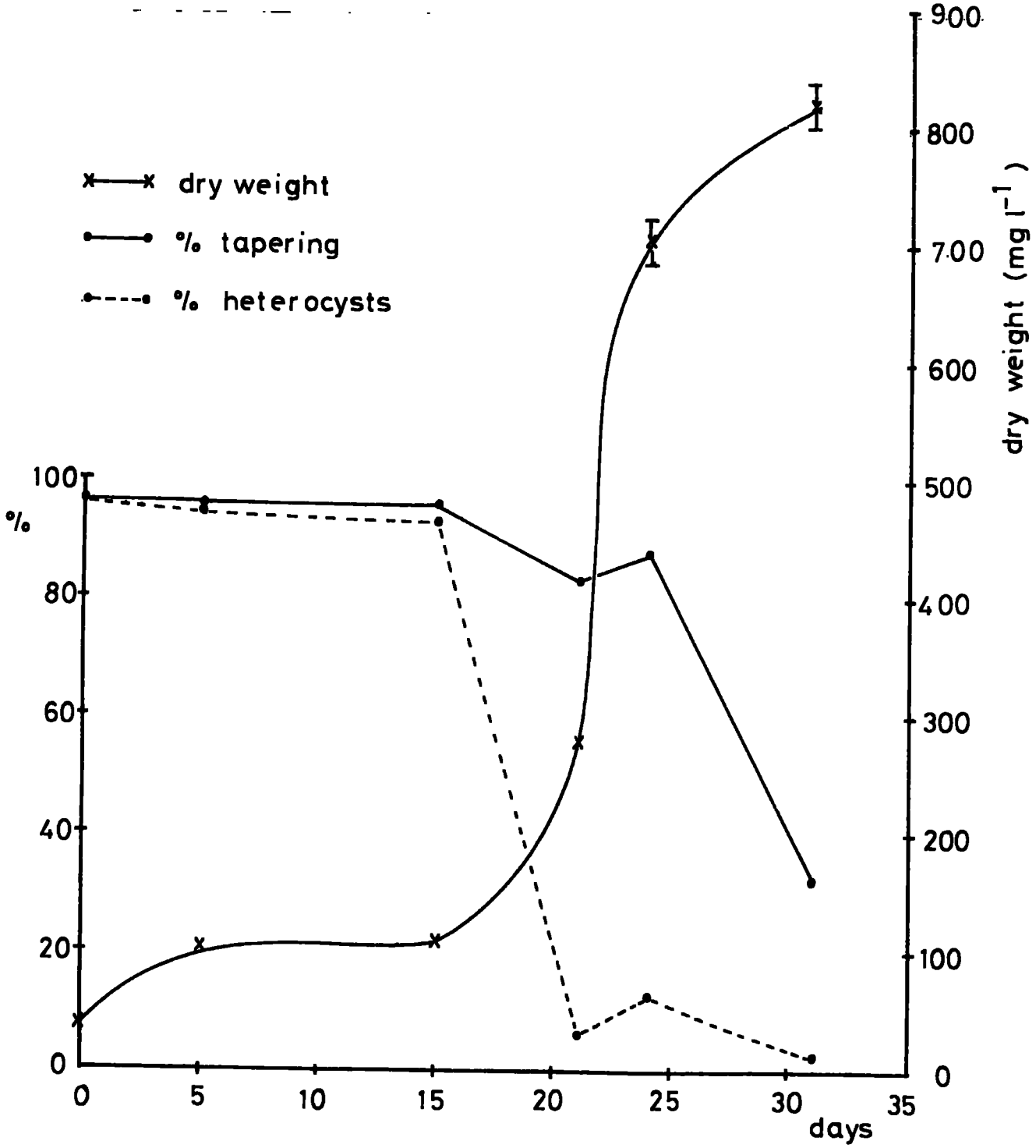


Fig. 4.12 Growth and morphology of Calothrix scopulorum in + NO_3-N medium

a marked decrease in the heterocyst frequency at an early stage of growth, while a decrease in the frequency of tapered trichomes occurred at a later stage. As in the case of C. viguieri, the absolute decrease in the frequency of heterocysts was greater than the decrease in the frequency of tapering.

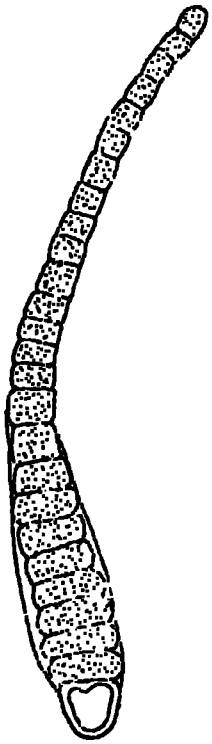
Fig. 4.13 illustrates changes in the morphology of C. scopulorum in + N and - N media. This species did not develop a taper at both ends during any stage of growth. The mean basal and apical widths of C. scopulorum at various stages of growth are given in Table 4.6. Values of tapering, calculated using the four tapering indices, are given in Table 4.7. On and before day 15 all of the 30 trichomes measured were tapered. Tapering indices T_1 and T_2 suggested that the absolute difference and the % difference between the cells with maximum width and those with minimum width, were greater in the + N medium. However, using T_3, which takes trichome length into consideration, the trichomes in the + N medium appear to be less tapered. This may be attributed to the influence of length on this index of tapering.

On and after 21 days growth, all of the indices of tapering indicated that trichomes cultured in + N medium were less tapered. As a check that morphological changes were not brought about by changes in ionic concentration, material was cultured in - N medium with NaCl-Na at the same concentration as $NaNO_3$ in the + N medium. The trichomes cultured in the presence of NaCl were similar in appearance to those cultured in - N medium.

Results of acetylene reduction assays of C. scopulorum in + N and - N media are given in Fig. 4.14 and Tables 4.8, 4.9.

(a)

- N_2 21 days growth



(b)

+ N_2 21 days growth

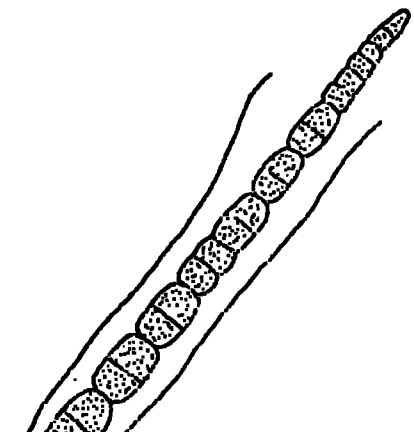


Fig. 4.13 Morphology of Calothrix scopulorum in - NO_3-N and + NO_3-N media

Table 4.6 Basal and apical widths of *Calothrix scopulorum* cultured in ± NO_3-N media.

(measurements in μm)

	Basal width		Apical width	
Day	- N	+ N	- N	+ N
5	7.37 ± 0.16	7.15 ± 0.14	2.93 ± 0.46	2.63 ± 0.94
15	8.35 ± 1.53	9.47 ± 1.65	2.83 ± 0.37	2.95 ± 0.65
21	8.20 ± 1.93	6.10 ± 0.94	3.25 ± 0.56	3.90 ± 1.20
24	9.05 ± 0.72	8.48 ± 1.80	2.57 ± 0.50	3.93 ± 1.32
31	8.05 ± 1.57	5.80 ± 0.93	2.92 ± 0.45	4.45 ± 1.06

Table 4.7 Change in the tapering of *Calothrix scopulorum* cultured on + NO_3-N and - NO_3-N media. T_1, T_2, T_3 and T_4 are tapering estimates

age in days	T_1		T_2		T_3		T_4		Number of trichomes, out of 30, with no taper	
	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N
5	4.4 ± 0.3	4.3 ± 0.3	58.6 ± 1.9	60.0 ± 2.5	-	-	-	-	0	0
15	5.5 ± 0.3	6.5 ± 0.4	65.3 ± 1.6	67.2 ± 2.3	8.5 ± 0.7	5.7 ± 0.4	103.0 ± 8.3	58.7 ± 3.6	0	0
21	5.0 ± 0.4	2.2 ± 0.3	58.6 ± 2.3	34.7 ± 3.9	7.8 ± 0.7	3.3 ± 0.4	91.4 ± 5.8	52.3 ± 6.2	0	6
24	6.5 ± 0.2	4.5 ± 0.4	71.4 ± 1.2	52.3 ± 3.3	9.1 ± 0.6	5.6 ± 0.5	99.2 ± 5.4	64.7 ± 4.9	0	0
31	5.1 ± 0.3	1.3 ± 0.2	62.1 ± 0.2	22.2 ± 4.0	8.0 ± 0.7	1.7 ± 0.4	101.5 ± 5.9	27.8 ± 5.9	0	13

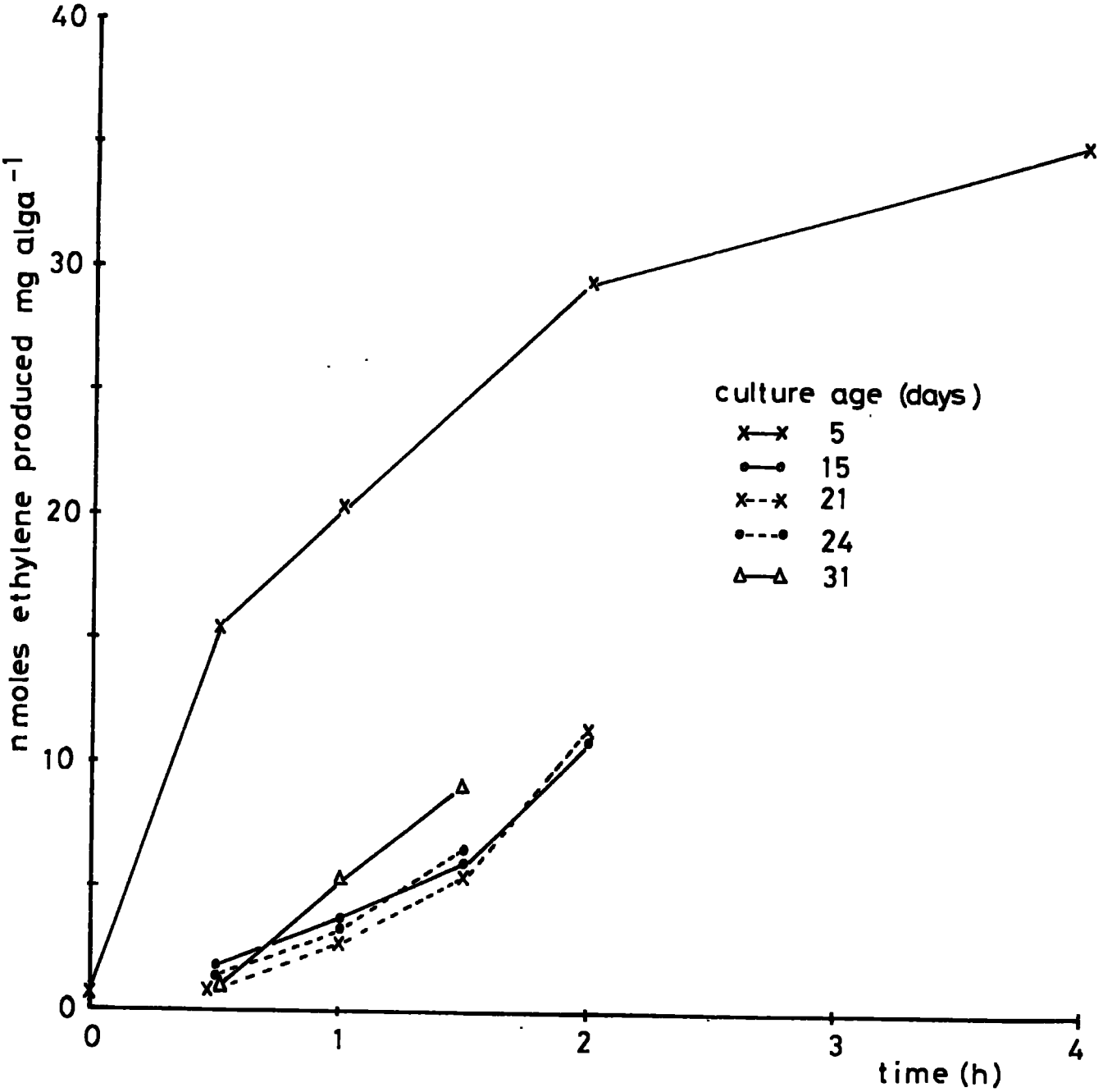


Fig. 4.14 Acetylene reduction assay of Calothrix scopulorum cultured in -NO_3-N medium

Table 4.8 Acetylene reduction assay of Calothrix scopulorum cultured in - NO_3-N medium.

total nmoles C_2H_4 produced mg alga^{-1}

days growth / incubation time (h)	5	15	21	24	31
0	0.58 ± 0.0	-	-	-	-
0.5	15.58 ± 4.7	1.54 ± 0.1	0.92 ± 0.1	1.78 ± 0.3	1.21 ± 0.1
1.0	20.46 ± 2.7	3.82 ± 0.1	2.99 ± 0.7	3.36 ± 0.6	5.41 ± 1.1
1.5	-	6.09 ± 1.1	5.73 ± 0.8	6.40 ± 0.7	9.12 ± 2.8
2.0	29.43 ± 11.9	10.89 ± 1.1	12.36 ± 0.5	-	-
4.0	35.20 ± 14.8	-	-	-	-

Table 4.9 Acetylene reduction assay of Calothrix scopulorum cultured in + NO_3-N medium.

nmoles C_2H_4 produced mg $alga^{-1}$

days growth	5	15	21	24	31
incubation time (h)					
0	1.16 ± 0.0	2.67 ± 0.8	6.24 ± 0.1	2.32 ± 0.1	3.38 ± 0.1
2	1.52 ± 0.4	2.29 ± 0.4	1.28 ± 0.0	2.17 ± 0.1	3.82 ± 0.2

After 5 days of growth in - N medium, there was a high rate of acetylene reduction, but this decreased rapidly and remained at a constant low level throughout the rest of the growth period. The material cultured in the + N medium had a low rate of acetylene reduction, which was however slightly higher than the rate recorded for C. viguieri, in + N medium.

4.4 Discussion

The results obtained when C. brevissima and C. viguieri were cultured in the presence of NH_4-N are in agreement with those of Fay, Stewart, Walsby and Fogg (1968), in that at relatively high initial concentrations of NH_4-N, tapering and heterocysts did not develop. At the highest levels of NH_4-N used, a high proportion of the trichomes of both strains were parallel and fairly long. At these high levels of NH_4-N, some of the filaments of C. viguieri also developed a Hammatoidea-like appearance. Instead of tapering towards one end, the trichomes tapered towards both ends, being widest in the central region. The sheath also tapered towards both ends. This type of morphological variation was not observed in trichomes of C. brevissima. (Before the influence of the higher levels of NH_4-N can be studied in more detail, problems of buffering must be overcome).

As a result of culturing C. viguieri and C. scopulorum in the presence of NO_3-N the proportion of trichomes with heterocysts and which tapered also decreased. In the case of C. viguieri there

was a noticeable decrease in the percentage of trichomes with heterocysts after 12 days growth. In contrast the percentage of trichomes which tapered decreased gradually throughout the growth period. This response may be compared with that shown in the presence of NH_4-N, in which decreases in both the percentage of trichomes which had heterocysts and the percentage which tapered, followed more similar trends. In quantitative terms there was a greater reduction of tapering frequency in NH_4-N medium than in NO_3-N medium. At the higher level of NO_3-N, C. viguieri developed the Hammatoidea-like appearance which had been observed in medium with NH_4-N. Some of the filaments were parallel, but were not as long as those in medium + NH_4-N.

In the case of C. scopulorum cultured in the presence of NO_3-N, there were similar decreases in the percentages of trichomes which tapered and possessed heterocysts. The decrease in heterocysts occurred fairly rapidly after 15 days growth. However, although the decrease in tapering also occurred rapidly (compared to that in C. viguieri) it did not occur until after 24 days growth. Trichomes of C. scopulorum in medium + NO_3-N remained fairly short, some became parallel but others possessed a slight taper. No trichomes with a Hammatoidea-like appearance were observed.

The nitrogen-fixing abilities of C. viguieri and C. scopulorum in + N and - N media have been studied using the acetylene reduction assay. Values obtained are quoted here as nmoles C_2H_4 produced mg $alga^{-1}h^{-1}$. For an indication of the absolute value of nitrogen fixation the theoretical conversion factor of 3 (C_2H_4 produced: N_2 fixed), suggested by Stewart, Fitzgerald and Burris (1968) may be used.

In NH_4-N *C. viguieri* had a lower rate of nitrogen-fixation at the higher levels of combined nitrogen, the rates measured being 20 nmoles C_2H_4 produced mg $alga^{-1}$ h^{-1} at 0 mg l^{-1} NH_4-N and 0.67 nmoles C_2H_4 produced mg $alga^{-1}$ h^{-1} at 14.0 mg l^{-1} NH_4-N. A comparison of the nitrogen-fixation in + NO_3-N showed that in + N medium, the rate of fixation was low throughout the growth period. In - N medium, the rate of fixation increased from 10.1 to 24.7 nmoles C_2H_4 produced mg $alga^{-1}$ h^{-1}, between 5 and 12 days after inoculation, and following this decreased gradually. In the case of *C. scopulorum*, the rate of nitrogen fixation was initially high (20.5 nmoles C_2H_4 produced mg $alga^{-1}$ h^{-1}), but after 15 days growth it had fallen to a fairly consistent low level (3.0 - 5.4 nmoles C_2H_4 produced mg $alga^{-1}$ h^{-1}). Jones and Stewart (1969a) reported high initial rates of nitrogen fixation when transfering *C. scopulorum* to different environmental conditions. In medium + NO_3-N, the values of nitrogen fixation were low, but were slightly higher than those recorded for *C. viguieri* under similar conditions.

The changes in nitrogen fixation observed in the presence of NH_4-N may be interpreted in terms of a decrease in the percentage of trichomes with heterocysts. In NO_3-N the decrease in nitrogen fixation of *C. scopulorum* occurred at a similar time to the reduction in the percentage of trichomes with heterocysts. However, in *C. viguieri* the two trends were not similar, there being a fairly marked decrease in the percentage of filaments with heterocysts but a gradual decline in the amount of nitrogen fixed.

The tapering indices, described in Section 3.21, were used to provide a quantitative record of changes in morphology in NO_3-N media. In all cultures of C. viguieri which were more than 18 days old, the results indicated a decrease in tapering in + N media. In C. scopulorum there is an overall indication of a decrease in tapering in + N medium, however the situation is complicated by the fact that within any 30 trichomes taken at random, a considerable range of developmental stages and hence lengths, may be present (Section 3.21). In retrospect this appears to be one of the major problems in the study of tapering in quantitative terms. It seems necessary to take such variation into account or to proceed to a detailed quantitative examination of each stage.

5 THE DEVELOPMENT OF AN OBJECTIVE, SIMULTANEOUS KEY FOR THE IDENTIFICATION OF CALOTHRIX AND RIVULARIA

5.1 Introduction

The fact that the wide range of morphological variation shown by the blue-green algae produces many taxonomic problems has been pointed out in Section 1.2. When it was necessary for the present author to assign a name to members of the genera Calothrix and Rivularia, many difficulties were encountered. The main reasons for this were firstly, the great range in morphological variation even of the characters used in the floras and secondly, the unknown influence of the environment on that variation.

The three main floras used during the present work, were those of Tilden (1910), Geitler (1932) and Desikachary (1959). The work used mainly by western European workers is that of Geitler (1932); although Elenkin's Russian flora (1936) may contain even more information. Descriptions from the three floras used during the present work agreed on most points, although there were several discrepancies concerning the calcification of the colonies. Geitler (1932) described R. minutula as being encrusted, while Tilden (1910) stated that it was not calcified. Furthermore, despite describing the difficulties of using calcification as a character on which to base identification of Rivularia, Geitler (1932) used it in his flora. In the section of his flora dealing with Calothrix only 28 of the 69 species described are included in the key. The short-comings of assigning specific names based on morphological characters alone, are often appreciated, although it is essential to do this in order to compare results with other workers.

In an attempt to overcome some of the problems described above, an objective, simultaneous key (Sneath and Sokal 1974) was devised (Section 5.41). In order to do this it was necessary to develop a standard procedure for recording the data in a form which could be processed by computer (Section 5.2, 5.3). Once the information for the key had been collected it was possible to use it with a computer program 'QUESTION', to obtain information about the relationships between morphological characters (Section 5.42).

5.2 Development of a standard recording procedure

The author translated the descriptions from Geitler (1932) and by collecting this and other information together, it was possible to become acquainted with the range of characters shown by Rivularia and Calothrix. These data were then used to devise a standard recording procedure, which could be used for identification purposes and for answering questions about morphological characters of the group. It was also hoped that this scheme would encourage future workers to record full descriptions, in a uniform manner and in a semi-quantitative way at least.

The information collected from the literature is given in Appendix II and the authorities for each species in Appendix I. The main sources of data were the floras quoted in Section 5.1. The following papers, some of which contain original descriptions and some which describe particular species in detail, were also used: Bornet and Flahault (1886-1888), Frémy (1929-1933), Frémy (1931), Gomont (1895), Gonslaves and Kamat (1960), Paplik (1946), Rao (1937), Setchell (1895), Swellengrebel (1910), Webber (1967) and Womersley (1946). When data were ambiguous or missing, attempts were made to obtain original descriptions; however this was not always possible.

Based on the information available and the author's observations on field material, a list of characters and their subdivisions were drawn up (Section 5.21). The term "character" has been used here in the sense of being a property of an organism which can be expressed in a number of different ways or 'character categories' (Lockhart and Liston 1970). Character categories are equivalent to the character states of Sneath and Sokal (1974).

Sokal and Sneath (1964) gave a detailed discussion of the choice of characters and groups of "inadmissible characters". The latter include meaningless characters such as specimen code numbers and correlated characters. They also stressed that each character category should be mutually exclusive. One of the major problems in devising a standard recording scheme, was delimiting each of the character categories. Where possible this was based on formal definitions. Although some of the character categories used by the author, can be arranged in a series, in which the two extreme categories are less similar than two neighbouring categories, no attempt has been made to force characters into such a series. For instance in the case of heterocyst shape it is impossible to arrange the categories in a series. This is an important fact which must be borne in mind when choosing a coefficient of resemblance, as discussed in Section 5.41. Unfortunately coding, particularly of field material, is complicated by the frequent presence, in one colony, of material displaying several distinct states of a single character. In such cases it is suggested that a record be kept of the fact, however, for coding purposes it is necessary to choose a single category for each character. This 'choice' of character categories is outlined

in a guide to coding characters (Section 5.22). As far as possible the most 'advanced-condition' is coded. For example the hormogonia have a pale sheath which may become pigmented later, and in these cases the deepest colour of the sheath is recorded. As it was possible to record some additional information in quantitative terms when field material was collected, slight modifications were made to this recording scheme (Section 5.23).

Characters which are used for identification purposes should be (a) easily observable (b) show relatively little variation and (c) change in a fairly easily definable way with changes in the environment or with age. With reference to the recording scheme used here, most of the characters are easily observable, although it may be difficult to determine the arrangement of trichomes in the genus Calothrix. Characters 12 and 18, the shape of the basal cell and the shape of the heterocyst respectively, show considerable variation. Characters 5, 7 and 20, calcification, the texture of the colony and texture of the sheath respectively, also show variation with environmental factors. The omission of these characters, when identifying species, is considered in Section 5.411.

5.21 Standard recording procedure for describing the genera Calothrix and Rivularia (guide to recording procedure given in Section 5.22)

(1) Physiognomic form

0. Not known
1. Colony spherical
2. Colony hemispherical or caespitose
3. Colony expanded or lobed

4. Thallus fasciculate or penicillate

5. Crust or film

9. Not applicable

(2) Colony height or filament length

0. Not known

1. ≤ 0.5 mm

2. > 0.5 ≤ 1 mm

3. > 1 ≤ 2 mm

4. > 2 ≤ 4 mm

5. > 4 ≤ 8 mm

6. > 8 ≤ 16 mm

7. > 16 mm

(3) Colonies confluent or not

0. Not known

1. Colonies confluent

2. Colonies not confluent

9. Not applicable

(4) Colony or thallus hollow or solid

0. Not known

1. Colony or thallus solid

2. Colony or thallus hollow

(5) Calcification

0. Not known

1. Abundant

2. Moderate or slight

3. Absent

(6) Distribution of calcium carbonate

0. Not known
1. Crystals in definite zones
2. Crystals in central region of colony or close to sheath
3. Crystals scattered
9. Not applicable

(7) Texture

0. Not known
1. Hard or firm
2. Dry
3. Soft or gelatinous

(8) Arrangement of trichomes

0. Not known
1. Radial, closely appressed
2. Parallel and/or erect, closely appressed
3. In bundles, closely appressed
4. Parallel and close to the ground, closely appressed
5. Radial and loosely appressed
6. Parallel and erect, loosely appressed
7. Bundles and loosely appressed
8. Irregular and intertwined, often close to ground

(9) Tapering

0. Not known
1. Trichome tapers gradually from base to apex
2. Trichome narrower towards base, widening and then tapering towards apex
3. Abrupt taper, often near apex
4. Only slight tapering, possibly in end cell only
9. Not applicable

(10) Hair

0. Not known
1. Hair long
2. Hair short
3. Hair present but no details recorded
4. Hair absent

(11) Cell width at base of trichome

0. Not known
1. Cell width ≤ 4 µm
2. $> 4 \leq 8$ µm
3. $> 8 \leq 16$ µm
4. > 16 µm

(12) Shape of basal cell

0. Not known
1. Cells at base shorter than broad
2. Cells at base as broad as long
3. Cells at base longer than broad

(13) Base of trichome swollen or not

0. Not known
1. Base swollen
2. Base not swollen

(14) Cell indentations

1. All cells indented at crosswalls
2. Some cells indented at crosswalls
3. Cells not indented

(15) Width of basal cell compared to heterocyst

0. Not known
1. Basal cell wider
2. Basal cell and heterocyst width the same
3. Heterocyst wider

(16) Number of heterocysts

0. Not known
1. Single
2. More than one
9. Not applicable

(17) Position of heterocysts

0. Not known
1. Basal only
2. Basal plus > 2 intercalary heterocysts
3. Without basal heterocyst(s), but > 2 intercalary
4. Basal plus < 2 intercalary
5. Without basal, < 2 intercalary heterocysts

(18) Shape of basal heterocyst

0. Not known
1. Width = length
2. Width > length
3. Width < length
4. Any combination of 1, 2 and 3

(19) Shape of sheath

0. Not known
1. Distinctly diverging or funnel-shaped
2. Distinctly attenuated and sometimes extending beyond trichome
3. Parallel and/or close to trichome throughout

(20) Texture of sheath

0. Not known
1. Firm or hard
2. Gelatinous

(21) Width of sheath

0. Not known
1. ≤ 2 µm
2. $> 2 \leq 4$ µm
3. $> 4 \leq 8$ µm
4. > 8 µm

(22) Colour of sheath

0. Not known
1. Yellow-brown
2. Yellow in parts
3. Pale
4. Colourless
5. Other

(23) Sheath lamellations

0. Not known
1. Conspicuous
2. Indistinct
3. Absent

(24) Sheath lacerations

0. Not known
1. Present
2. Absent

(25) Spores

0. Not known
1. Present
2. Absent

(26) Habitat

0. Not known
1. Marine
2. Freshwater
3. Marine and freshwater

5.22 Guide to the standard recording procedure

A detailed guide to the use and application of each character category has been provided, to reduce as much as possible the subjective element in describing organisms. In the allocation of any character category, conditions which represent the most advanced stage of growth take precedence.

(1) Physiognomic form

Category 1.2 includes a considerable range in sizes, from colonies which are easily visible to those which are only just visible macroscopically.

Category 1.5 has the appearance of a definite crust or film, but other algae may be intermingled with the Rivulariaceae. This category is used for any macroscopic growth form which is not included in categories 1.1-1.4.

Category 1.9 implies that the algal filaments are scattered in the mucilage of other algae, or that the filaments are sparsly distributed or scattered, possibly because an area has only recently been colonised.

(2) Colony height or filament length

As the growth form may be variable, measurements of colony height are taken from the thickest point of growth i.e. the radius of a spherical colony and the maximum thickness of other growth forms. If the colony is not macroscopic, and/or only filament lengths are available, these are coded.

(3) Colonies confluent or not

This character is applicable to categories 1.1-1.3.

Category 3.1 is recorded if colonies are sometimes confluent.

Category 3.2 is recordedif no information is given for growth forms in the categories 1.1-1.3.

(4) Colony of thallus hollow or solid

This character applies to character categories 1.1-1.5.

Category 4.2 applies to growth forms which are hollow at some stage in the life cycle.

(5) Calcification

Category 5.1 applies if at least $\frac{2}{3}$ of the material is calcified and/or this character is definitely noticeable when mounting material for microscopic examination.

Category 5.2 applies if calcification is not noticeable until the material is examined microscopically, or the character is not stressed in the floras.

This character must be used with caution, bearing in mind that calcification may vary with age and size of colony, substrate and water chemistry.

(6) Distribution of calcium carbonate

Category 6.1 is only used if zones are present. This character may be related to colony age or to some rhythm of growth.

Category 6.3 applies if there are no details of the distribution of the calcium carbonate and if the growth form is a calcareous crust.

(7) Texture

Although this character may provide a practical guide to distinguishing certain strains, the term itself covers several concepts. A hard texture may result from the growth form being calcified and therefore "hard or stony", or properties of the mucilage may produce a firm exterior to the colony. Alternatively a colony may be described as soft if the mucilage is soft, or if the material is not calcified.

Category 7.1 applies to all material which is difficult to squash under a coverslip.

Category 7.3 applies to all material which is easily squashed and if no information is given.

(8) Arrangement of trichomes

Trichomes are assumed to be loosely appressed unless described otherwise.

Trichomes from spherical or hemispherical colonies are assumed to be arranged radially. Geitler (1932), reported that in Rivularia biasolettiana, the trichomes became more parallel in older colonies, and it is possible that this could happen in other strains.

(9) Tapering

Unless stated otherwise, it is assumed that trichomes taper gradually from the base to the apex.

Category 9.9 applies to hormogonia.

(10) The hair

Category 10.1 applies if > 50% of the trichome is hair.

(11) Width at base of trichome

If the maximum width is just above the base, measurement of the maximum width is recorded.

(12) Shape of basal cell

This character may show considerable variation.

If cells are described as being as long as or longer than wide, then longer than wide takes precedence, as it implies growth.

If cells are described as being as long as wide or shorter than wide, then shorter than wide takes precedence. This decision has

been based on observations which suggest that some trichomes swell at the base as they mature. The short length of the basal cell does not seem to be related to a basal meristem.

(13) Base of trichome swollen or not

Category 13.2 applies if no information is given.

(14) Cell indentations

This character describes indentations at the cross-walls of adjacent cells, in regions other than the meristem.

If not recorded assume absent.

(15) Width of basal cell compared to heterocyst

The maximum width of the basal cell is compared with the maximum width of the heterocyst.

Category 15.2 is used if the widths are described as almost equal.

(16) Number of heterocysts

This character refers to the number of basal and intercalary heterocysts.

Category 16.1 is used if only one heterocyst is obviously healthy.

(17) Position of heterocysts

'Abundant' refers to more than two.

If no information is given, the heterocyst is assumed to be basal.

(18) Shape of heterocyst

The heterocyst shape may be extremely variable within one species. As the factors which influence heterocysts shape are not known, the shape has been simplified to a relationship between the maximum width and the length.

(19) Shape of sheath

(20) Texture of sheath

(21) Width of sheath

Category 21.1 is used if the sheath is described as 'thin'.

(22) Colour of sheath

The darkest colour reported is recorded.

(23) Sheath lamellations

If not recorded assume absent.

(24) Sheath lacerations

If not recorded assume absent.

(25) Spores

If not recorded assume absent.

(26) Habitat

Category 26.3 is used for soil habitats.

5.23 Standard recording procedure applied to field material

Material collected in the field was coded in a similar way to the information from the literature. However as several additional aspects of trichome morphology could be recorded from the living material, slight modifications were made to the scheme outlined above.

The first eight character categories were the same as those given in Section 5.21. Following this are records of the abundance of different filament types within one colony i.e. character categories 9-12 for the field data were:

(9) Abundance of hormogonia (short parallel trichomes with an inconspicuous sheath and no heterocyst).

(10) Abundance of parallel trichomes (long parallel trichomes with an obvious sheath, with or without a heterocyst).

(11) Abundance of tapered trichomes.

(12) Abundance of dead and unhealthy trichomes.

Abundances were recorded on a subjective 1-5 scale. For computational purposes these 12 categories were designated A1-L1 (Section 2.11).

22 characters, designated A2-V2 (Section 2.11), were used for recording trichome data of field material. As mentioned above, several additional characters, which were not recorded for data from the literature, were recorded for the field data, namely characters B2, C2, D2 and F2. Apart from these, characters correspond to those given in Section 5.21. The 22 characters are as follows:

A2 Tapering - an additional category (5) has been added. This is coded when long parallel trichomes are present.

B2 Appearance of cells at apex of trichome - This is a new character, introduced to record additional information. This information has not been incorporated into the key. The categories are as follows:

(0) Not known

(1) Similar to those throughout the trichome

(2) With intrathylakoidal vesicles

(3) Colourless and forming a hair

The next three characters were designed to record useful information about the field material in quantitative terms.

C2 Length of hair - recorded in μm

D2 Length of trichome - recorded in μm

E2 Maximum width of trichome - recorded in μm

F2 Shape of basal cell

G2 Basal cell swollen or not

H2 Cell indentations

I2 Width of basal cell compared to heterocyst - category 9 was added to indicate 'not applicable'

J2 Number of heterocysts

K2 Position of heterocysts

L2 Shape of basal cell

M2 Heterocysts width-recorded in μm

N2 Heterocyst length - recorded in μm

O2 Shape of sheath

P2 Texture of sheath

Q2 Width of sheath - recorded in μm

R2 Sheath colour

S2 Sheath lamellations

T2 Sheath lacerations

U2 Spores

V2 Habitat

The information collected was stored as part of the program 'FIELD' (Section 2.11). The relevant characters were readily extracted and used with the 'IDENTIFY' and 'QUESTION' programs (Section 2.11).

As the author's familiarity with the Rivulariaceae is restricted to relatively few genera, the list of characters and their categories is not assumed to be comprehensive. It is to be hoped that other genera could be incorporated into this scheme and furthermore, that

the character categories could be assigned as a result of experimental investigations. In this way the subjective element in assigning character categories would be reduced further.

5.3 Coding the data

Data have been recorded as qualitative multistate characters. The code numbers used for the character categories are between 0 and 9. In agreement with other algal recording schemes used at the Department of Botany, Durham, category 0 is kept for data which are 'not known' and category 9, for data which are 'not applicable'. It is important to bear in mind the difference between the two responses. Data may be missing because they are 'not known' as in the following two examples:

(a) In considering the question of presence or absence of a spore, there may be insufficient information to determine whether, under specific (but possibly unknown) circumstances, the spore could be present; and such data would be coded as 'not known'.

(b) Characters may be obscured or missing because the specimen has been damaged; and the data would be coded as 'not known'. Data may also be missing because they are 'inapplicable', for example, questions concerning morphological characters of spores are inapplicable if the spores are absent.

Once the data were coded they were stored on punch cards. The data were punched in F2 format (i.e. blank, number, blank, number, etc.), details being given in Section 2.11.

5.4 Computer analysis of the data

As described previously, the data were used for identification purposes and for answering questions about the morphological characters of the species. The techniques used and results obtained are described in Sections 5.41 and 5.42 respectively.

5.41 Identification

The main reason for collecting the data was to use all of the information about each species of Calothrix and Rivularia, in an objective identification. This type of key has been called a 'simultaneous key' (Sneath and Sokal 1974). The technique involves comparing each unknown species in turn, with each known species, in the hope of obtaining an objective (but not necessarily unique) identification. The species showing the closest agreement with the unknown was taken as the correct identification.

Any resemblance measure can be used to assess the best match (Sneath and Sokal 1974). Of the many coefficients of resemblance discussed by them, association coefficients appear to be the most suitable for qualitative multistate data. The fundamental formula for a coefficient of association consists of a number of matches divided by a term implying the possible number of comparisons, but varying in detailed composition (Sokal and Sneath 1964). The formulae quoted by Sokal and Sneath (1964) recognise only two types of comparisons, which result in (i) matches between data, designated "m" and (ii) mismatches between data, designated "u". These formulae do not take into account the possibility of an 'unknown' or 'not recorded' result being included. In the data collected by the author

the following four types of comparisons were recognised:

(i) genuine matches e.g. 3-3, designated m

(ii) false matches e.g. 0-0 or 9-9, designated m'

(iii) genuine mismatches e.g. 3-2, designated u

(iv) false mismatches e.g. 0-3 or 9-3, designated u'

The simplest association coefficient is the simple matching coefficient (S_{SM}) of Sokal and Michener (1958). This was described by Sokal and Sneath (1964) as:

$$S_{SM} = \frac{m}{n} = \frac{m}{m+u}$$ (where n = number of possible matches)

For present purposes, it was felt that false matches and false mismatches should not be included in the estimate of resemblance. The coefficient S_{SM} was therefore calculated as $\frac{m}{m+u}$, using the author's notation, given above. In two other coefficients quoted by Sokal and Sneath (1964), m' and u' are not included. These coefficients are:

(a) an unnamed coefficient (S), which gives matches twice the weight of mismatches $S = \frac{2m}{2m+u}$

(b) The coefficient of Rogers and Tanimoto (1960), S_{RT} which may be written $S_{RT} = \frac{m}{m+2u}$

This gives unmatched pairs twice the weight of matched pairs. A coefficient, designated here as S_K was also used. This estimated the value of the number of true matches divided by the total number of

variables and can be represented by $S_K = \frac{m}{m+m' + u+u'}$

These coefficients were used in the program 'IDENTIFY', a copy of which is given in Section 2.11. The program was used first to check whether any of the known species would "key down" to any of the other known species, using a simultaneous key. At the same time it was also possible, using this objective technique, to make an appraisal of comments from the literature, that a certain species closely resembled one another (Section 5.411). Secondly, the program was used to 'identify' unknown species. The majority of these were species collected in the field however, the three strains of Calothrix used for experimental work and several new strains and varieties were also keyed-down (Section 5.412).

It is important to bear in mind that when using data of the type used here, a measure of agreement can be based on one of two considerations (Sokal and Sneath 1964). Firstly, a match between two character states of a multistate character may be considered equal to that of two-stage characters. In such a case the simple matching coefficient S_{SM}, is extended to multistate characters. Such an estimate of resemblance however, makes no allowance for the possibility that a match in a five-state character may be less likely than a match in a two-state character. The second approach considers the probability of a given match taking place, reasoning that matches in rare character states should count more heavily than those of common character states. The latter approach immediately encounters the problem of determining which character states are rare, and hence the former approach has been adopted here.

5.411 Keying-down known species

Every species listed in Appendix II was used in turn as the unknown species, and identified using the coefficients S_{SM} and S_K.

As stated in Section 5.41, there are several comments in the literature implying that a certain species closely resembles another species. A list of the species concerned is given in Table 5.1, the data being taken from Frémy (1931), Geitler (1932), Malmeström (1972), Setchell (1896) and Setchell and Gardner (1903). The information from the above literature can be compared with the results obtained using 'IDENTIFY' (Table 5.2).

The results indicate that using S_{SM}, two pairs of species were identical (1) C. atricha and C. scytonemicola and (2) C. evanescens and C. membranacea. Using S_K, only C. evanescens and C. membranacea were identical. (It should be pointed out that using the three coefficients S_{SM}, S, and S_{RT} gave the same results for the nearest neighbours, although the actual values of the coefficients differed. For this reason, only the results obtained using the simplest coefficient S_{SM} and S_K will be considered further).

A comparison of Tables 5.1 and 5.2 shows that five of the nearest neighbours obtained using S_{SM} and the simultaneous key were the same as those described in the literature. These were:

1. C. aeruginea and C. confervicola
2. C. conica and C. marchica
3. C. bharadwaja and C. gracilis
4. C. scopulorum and C. confervicola
5. C. pilosa and C. vivipara

Table 5.1 List of species described in the literature as 'closely resembling each other'

Calothrix adscendens	and	*C. confervicola*
C. aeruginea	"	*C. confervicola*
C. africana	"	*C. parietina*
C. antarctica	"	*C. sandviencis*
C. atricha	"	*C. conica*
C. atricha	"	*C. marchica*
C. bharadwaja	"	*C. fritschii*
C. bharadwaja	"	*C. gracilis*
C. bossei	"	*C. adscendens*
C. calida	"	*C. kuntzei*
C. castellii	"	*C. confervicola*
C. castellii	"	*C. pulvinata*
C. consociata	"	*C. confervicola*
C. consociata	"	*C. scopulorum*
C. contarenii	"	*C. scopulorum*
C. crustacea	"	*C. aeruginea* (when young)
C. fasciculata	"	*C. contarenii*
C. fasciculata	"	*C. scopulorum*
C. fusca	"	*C. parasitica*
C. gardneri	"	*C. gracilis*
C. juliana	"	*C. confervicola*
C. membranacea	"	*C. intricata*
C. parietina	"	*C. thermalis*
C. parva	"	*C. fusca*
C. prolifera	"	*C. crustacea*
C. pulvinata	"	*C. scopulorum*
C. viguieri	"	*C. breviarticulata*
C. vivipara	"	*C. pilosa*
C. vivipara	"	*C. scopulorum*
Rivularia bornetiana	"	*R. biasolettiana*
R. mamillata	"	*R. nitida*
R. nitida	"	*R. atra*

Table 5.2 Nearest neighbours for each known species using the coefficients of resemblance S_{sm} and S_k

known	nearest neighbour using S_{sm}	number of matches (m)	number of mismatches (u)	nearest neighbour using S_k	number of matches (m)
scendens	C. scytonemicola	14	3	C. scopulorum	14
				C. scytonemicola	14
ruginea	C. evanescens	13	6	C. confervicola	15
				C. floccosa	15
				C. membranacea	15
ruginosa	C. submarchica	14	4	C. submarchica	14
ricana	C. antarctica	13	4	C. antarctica	13
				C. gelatinosa	13
tarctica	C. africana	13	4	C. africana	13
	C. violacea	13	4	C. cylindrica	13
				C. doliochomeres	13
				C. floccosa	13
				C. violacea	13
richa	C. scytonemicola	14	0	C. bharadwaja	16
				C. elenkinii	16
radwaja	C. gracilis	18	3	C. gracilis	18
sei	C. submarchica	12	5	C. clavata	15
				C. clavatoides	15
unii	C. clavatoides	18	4	C. clavatoides	18
viarticulata	C. gelatinosa	10	4	C. gelatinosa	10
vissima	C. marchica	16	2	C. conica	17
lida	C. fulleborni	9	4	C. bharadwaja	13
	C. scytonemicola	9	4		
tellii	C. juliana	14	3	C. braunii	15
vata	C. minima	16	3	C. clavatoides	17
				C. kossinskajae	17
				C. membranacea	17
vatoides	C. submarchica	15	3	C. braunii	18
umbiana	C. minima	16	3	C. clavatoides	16
				C. elenkinii	16
				C. ghosei	16
				C. membranacea	16
				C. minima	16
ica	C. marchica	16	2	C. brevissima	17
fervicola	C. aeruginea	15	8	C. aeruginea	15
	C. scopulorum	15	8	C. scopulorum	15
sociata	C. parva	12	3	C. elenkinii	14
tarenii	C. parasitica	13	5	C. crustacea	16
stacea	C. fasciculata	16	6	C. contarenii	16
				C. fasciculata	16
				C. scopulorum	16
indrica	C. floccosa	17	5	C. floccosa	17
ortica	C. gardneri	10	7	C. aeruginea	13
	C. pilosa	10	7		
iochomeres	C. geitonos	20	2	C. geitonos	20
hellii	C. sphaerospora	12	3	C. juliana	13
nkinii	C. marchica	15	3	C. gracilis	17
	C. minuscula	10	2		
phytica	C. tenella	14	1	C. evanescens	14
				C. membranacea	14
				C. minima	14
				C. tenella	14
escens	C. membranacea	18	0	C. membranacea	18
iculata	C. prolifera	16	5	C. crustacea	16
				C. prolifera	16
ultii	C. evanescens	13	3	C. columbiana	14
				C. elenkenii	14
				C. ghosei	14
				C. kossinskajae	14
				C. membranacea	14
osa	C. cylindrica	17	5	C. cylindrica	17
borni	C. bharadwaja	11	3	C. atricha	11
	C. submarchica	11	3	C. bharadwaja	11
				C. clavatoides	11
				C. donnellii	11
				C. gracilis	11
				C. intricata	11
	R. manginii	13	3	C. submarchica	11
				C. doliochomeres	13
				C. gelatinosa	13
				C. parasitica	13
				C. stellaris	13
				R. globiceps	13
				R. manginii	13
-violacea	C. clavata	14	7	C. braunii	15
uri	C. clavata	12	4	C. clavata	12
	C. fusco-violacea	12	4	C. fusco-violacea	12
				C. linearis	12
nos	C. doliochomeres	20	2	C. doliochomeres	20
inosa	C. breviarticulata	13	4	C. bossei	14
				C. cylindrica	14
i	C. columbiana	16	4	C. columbiana	16
bcola	R. planctonica	14	2	C. doliochomeres	14
				C. elenkinii	14
				R. planctonica	14
si	C. kawraiskyi	10	3	C. kawraiskyi	10
lis	C. bharadwaja	18	3	C. bharadwaja	18
cata	C. marchica	16	1	C. membranacea	17
ica	C. simulans	14	2	C. marchica	14
				C. simulans	14
sa	C. castellii	14	3	C. castellii	14
	C. kawraiskyi	14	3	C. kawraiskyi	14
iskyi	C. simplex	16	3	C. simplex	16
nskajae	C. clavata	17	4	C. clavata	17
si	C. cylindrica	15	5	C. cylindrica	15
	C. parietina	15	5	C. parietina	15
ris	C. fulleborni	10	4	C. clavata	13
ica	C. intricata	16	1	C. brevissima	16
				C. conica	16
				C. intricata	16
nacea	C. evanescens	18	0	C. evanescens	18
	C. evanescens	16	2	C. membranacea	17
ula	C. elenkinii	10	2	C. elenkinii	10
tica	R. manginii	10	3	C. contarenii	13
				C. crustacea	13
				C. fusca	13
				C. scopulorum	13
ina	C. kuntzei	15	5	C. kuntzei	15
	C. consociata	12	3	C. antarctica	13

rigtina var. africana	C. prolifera	14	8
losa	C. desertica	10	7
	C. vivipara	10	7
olifera	C. fasciculata	16	5
lvinata	C. santapaui	11	5
menskii	C. robusta	9	4
busta	C. ramenskii	9	4
ndviencis	R. peguana	3	1
ntapaui	C. africana	11	4
opulorum	C. scytonemicola	12	5
ytonemicola	C. atricha	14	0
mplex	C. kawraiskyi	16	4
mulans	C. javanica	14	2
haerospora	C. donnellii	12	3
gnalis	C. scytonemicola	12	4
	C. wembaerensis	12	4
	R. aquatica	12	4
llaris	C. clavata	13	5
	C. fusca	13	5
marchica	C. clavatoides	15	3
	C. minima	15	3
nella	C. epiphytica	14	1
rmalis	C. evanescens	13	4
	C. marchica	13	4
uieri	C. bossei	14	6
lacea	C. epiphytica	13	1
ipara	C. sphaerospora	11	5
eri	C. membranacea	16	2
baerensis	C. scytonemicola	12	2
a	R. mamillata	18	6
atica	C. kawraiskyi	12	3
tralis	R. atra	13	7
cariana	R. haematites	15	4
solettiana	R. minutula	16	7
	R. rufescens	16	7
enlis	C. epiphytica	9	2
netiana	R. mamillata	14	6
	R. nitida	14	6
lata	R. mesenterica	15	6
a	C. submarchica	13	5
biceps	C. wembaerensis	14	3
matites	R. rufescens	17	4
girgi	R. peguana	4	2
iliata	C. geitonos	15	5
	R. atra	18	6
ginii	C. fusca	13	3
nterica	R. bullata	15	6
itula	R. rufescens	18	5
ida	R. peguana	4	2
ana	C. sandviencis	3	1
	C. scytonemicola	3	1
	C. weberi	3	1
ctonica	C. glaeocola	14	2
yotis	R. minutula	15	7
	R. nitida	15	7
	R. rufescens	15	7
scens	R. haematites	17	4
lardi	C. prolifera	12	4

C. consociata	12
C. kossinskajae	12
C. prolifera	14
C. desertica	10
C. vivipara	10
C. fasciculata	16
C. scopulorum	15
C. floccosa	13
C. confervicola	10
C. contarenii	10
C. doliochomeres	10
C. floccosa	10
C. adscendens	11
C. doliochomeres	11
C. africana	11
C. columbiana	11
C. doliochomeres	11
C. pulvinata	11
C. crustacea	16
C. adscendens	14
C. atricha	14
C. kawraiskyi	16
C. brevissima	14
C. columbiana	14
C. javanica	14
C. marchica	14
C. donnellii	12
C. clavatoides	15
C. gracilis	14
C. clavata	13
C. fusca	13
C. clavatoides	15
C. minima	15
C. membranacea	15
C. minima	15
C. membranacea	15
C. bossei	14
C. parietina	14
R. dura	14
C. doliochomeres	13
C. elenkinii	13
C. simplex	13
C. scopulorum	14
C. membranacea	16
C. doliochomeres	14
R. globiceps	14
R. mamillata	18
R. globiceps	14
R. planctonica	14
R. atra	13
R. nitida	13
R. haematites	15
R. mamillata	15
R. minutula	16
R. polyotis	16
R. rufescens	16
C. membranacea	11
R. dura	11
R. minutula	11
R. mamillata	
R. nitida	
R. mesenterica	15
C. braunii	16
R. planctonica	15
R. rufescens	9
C. stagnalis	9
R. mesenterica	
R. atra	18
R. globiceps	14
R. bullata	15
R. rufescens	18
R. polyotis	15
R. globiceps	5
R. manginii	5
R. nitida	5
C. braunii	15
R. globiceps	15
R. biasolettiana	16
R. minutula	17
C. prolifera	12

For clarity of representation specific names have not been underlined

Using the coefficient S_K, nine nearest neighbours were in agreement with the literature. These were 1-5 given above and -

6. C. fusca and C. parasitica
7. C. intricata and C. membranacea
8. C. scopulorum and C. pulvinata
9. C. vivipara and C. scopulorum

Both estimates of resemblance used with 'IDENTIFY' also suggested that Rivularia mamillata and R. atra were more closely related to each other than as was suggested by Geitler (1932), to R. nitida.

The number of matches, out of a maximum of 26 (Table 5.2) indicates that most 'nearest neighbours' to each Calothrix strain have between 12-16 matches and in the case of Rivularia 13-15 matches. The following 'nearest neighbours' of Calothrix strains had more than 16 matching characters:

C. cylindrica and C. floccosa (17 matches)

C. kossinskajae and C. clavata (17 matches)

C. bharadwaja and C. gracilis (18 matches)

C. braunii and C. clavata (18 matches)

C. evanescens and C. membranacea (18 matches)

C. doliochomeres and C. geitonos (20 matches)

The following 'nearest neighbours' of Rivularia had more than 15 matching characters:

R. biasolettiana and R. minutula (16 matches)

R. biasolettiana and R. rufescens (16 matches)

R. haematites and R. rufescens (17 matches)

R. mamillata and R. atra (18 matches)

R. minutula and R. rufescens (18 matches)

In comparison with this, several nearest neighbours had very few matching characters e.g.

C. sandviencis and R. peguana (3 matches)

R. peguana and C. sandviencis, C. scytonemicola or C. weberi (3 matches)

R. nitida and R. peguana (4 matches)

However, relatively little information was recorded about any of the above pairs and the impression that they form fairly distinct species should be treated with caution (see below).

Examination of 'nearest neighbours' using the similarity coefficient S_{SM} was also made. S_{SM} can range from 0, when no characters are identical, to 1.0 when all characters are identical. All of the pairs of species, listed above, with relatively high numbers of matches have relatively high values of S_{SM} (> 0.74). However, all of the pairs of species with low numbers of matches also have high values for S_{SM} (> 0.74), with the exception of R. nitida and R. peguana. Such findings emphasise the need for obtaining as much information as possible about each species, and the problems of relying on too few characters.

The 10 pairs of species with the lowest values of S_{SM} are:

C. desertica and C. gardneri	S_{SM}	0.59 (10 matches)
C. desertica and C. pilosa	S_{SM}	0.59 (10 matches)
C. pilosa and C. vivipara	S_{SM}	0.59 (10 matches)
C. parietina var. africana and C. prolifera	S_{SM}	0.64 (14 matches)
R. australis and R. atra	S_{SM}	0.65 (13 matches)
C. confervicola and C. aeruginea	S_{SM}	0.66 (15 matches)
C. confervicola and C. scopulorum	S_{SM}	0.66 (15 matches)
C. crustacea and C. fasciculata	S_{SM}	0.67 (16 matches)
C. fusco-violacea and C. clavata	S_{SM}	0.67 (14 matches)
R. nitida and R. peguana	S_{SM}	0.67 (4 matches)

It is evident that even if one species is fairly distinct, in that it has a low coefficient of similarity with its nearest neighbour, there is not always a single nearest neighbour.

5.412 Keying-down unknown material

The material collected from the streams visited during the ecological survey was identified using the coefficient S_{SM}, with both the complete set of characters and with the variable characters (Section 5.2) omitted. In order to keep the identification as objective as possible, every tapered trichome which had a different combination of characters was coded and identified. Each of these trichomes was also identified using the flora of Geitler (1932), the results obtained are shown in Table 5.3.

Table 5.3 Identification of field material (showing number of tapered trichomes keying down to each name)

sample number	Geitler (1932)	IDENTIFICATION USING 'IDENTIFY' with full list of characters	'IDENTIFY' with reduced list of characters
10001	Rivularia biasolettiana (6) R. dura (6) R. minutula (6)	R. beccariana (6)	R. beccariana (6)
10002	R. biasolettiana (8) R. dura (8) R. minutula (3)	R. beccariana (6) R. dura (2)	R. aquatica (2) R. beccariana (5) R. dura (2)
10003	R. biasolettiana (6) R. minutula (3)	R. aquatica (3) R. beccariana (1) R. borealis (2) R. dura (1)	R. beccariana (2) R. dura (3) R. planctonica (1)
10011	R. biasolettiana (4) R. dura (4) R. minutula (4)	R. aquatica (4)	R. aquatica (2) R. dura (3) R. rufescens (1)
10012	R. biasolettiana (5) R. dura (5) R. minutula (5)	R. aquatica (5)	R. aquatica (1) R. dura (4)
10013	R. biasolettiana (2) R. dura (4) R. minutula (4)	R. aquatica (4)	R. aquatica (2) R. dura (2) R. rufescens (1)
10014	R. biasolettiana (7) R. dura (7) R. minutula (4)	R. beccariana (6) R. viellardi (1)	R. beccariana (7) R. haematites (1) R. rufescens (2)
10015	R. dura (2)	R. dura (1) R. haematites (1) R. rufescens (1)	R. rufescens (2)
10016	R. dura (4)	R. beccariana (4)	R. aquatica (2) R. beccariana (1) R. dura (1)
10020	R. aquatica (8)	R. aquatica (8) R. globiceps (1)	R. aquatica (6) R. dura (2) R. globiceps (1) R. planctonica (1)
10021	R. aquatica (7) R. globiceps (1)	R. aquatica (6) R. globiceps (2)	R. aquatica (4) R. beccariana (3) R. haematites (3) R. rufescens (2)
10040	R. dura (5)	R. aquatica (1) R. beccariana (3) R. planctonica (3)	R. aquatica (1) R. dura (1) R. planctonica (2) R. rufescens (1)
10041	R. dura (6)	R. beccariana (6) R. planctonica (3)	R. aquatica (1) R. beccariana (1) R. haematites (1) R. planctonica (5) R. rufescens (2)
10042	R. dura (8)	R. beccariana (8) R. planctonica (3)	R. aquatica (3) R. planctonica (3) R. rufescens (2)
10043	R. dura (8)	R. aquatica (1) R. beccariana (1) R. planctonica (7)	R. aquatica (1) R. beccariana (1) R. planctonica (7)
10044	R. dura (7)	R. aquatica (1) R. beccariana (3) R. planctonica (2) R. rufescens (2)	R. aquatica (3) R. planctonica (4) R. rufescens (1)
10045	R. dura (5)	R. aquatica (1) R. planctonica (2) R. rufescens (2)	R. aquatica (3) R. planctonica (2)
10053	R. biasolettiana (9) R. minutula (7)	R. aquatica (9) R. planctonica (1)	R. aquatica (9) R. planctonica (1)
10056	R. dura (8)	R. aquatica (3) R. beccariana (4) R. dura (1)	R. aquatica (8)
10071	R. dura (9)	R. aquatica (1) R. beccariana (2) R. haematites (1) R. rufescens (5)	R. beccariana (3) R. rufescens (5) R. viellardi (2)

R. biasolettiana (4)
R. minutula (5)

R. dura (10)

R. dura (10)

R. dura (9)

R. dura (8)

R. dura (9)

R. dura (8)

R. dura (9)

R. dura (9)

R. dura (9)

R. haematites (10)

R. dura (8)

R. biasolettiana (2)
R. dura (4)
R. minutula (4)

R. biasolettiana (4)
R. dura (6)
R. minutula (5)

R. minutula (10)

R. dura (10)

R. dura (5)

R. biasolettiana (3)
R. dura (7)
R. minutula (5)

R. dura (10)

R. biasolettiana (8)
R. dura (10)
R. minutula (2)

R. biasolettiana (2)
R. dura (8)
R. minutula (8)

R. dura (10)

R. dura (8)

R. haematites (6)
R. rufescens (3)

R. beccariana (2)
R. biasolettiana (1)
R. rufescens (2)
R. viellardi (2)

R. beccariana (10)

R. beccariana (10)

R. beccariana (9)

R. biasolettiana (8)

R. biasolettiana (9)

R. biasolettiana (8)

R. beccariana (9)

R. beccariana (9)

R. beccariana (9)

R. aquatica (2)
R. beccariana (4)
R. haematites (4)

R. beccariana (1)
R. haematites (1)
R. rufescens (4)
R. viellardi (2)

R. aquatica (2)
R. beccariana (1)
R. dura (1)

R. aquatica (7)
R. beccariana (1)

R. aquatica (1)
R. beccariana (3)
R. dura (6)

R. aquatica (1)
R. beccariana (7)
R. dura (1)
R. haematites (1)

R. aquatica (2)
R. beccariana (6)
R. dura (1)

R. aquatica (5)
R. beccariana (1)
R. manginii (1)
R. rufescens (2)

R. beccariana (10)

R. beccariana (10)

R. beccariana (5)
R. biasolettiana (1)
R. dura (2)

R. beccariana (10)

R. beccariana (7)
R. rufescens (1)

R. haematites (9)

R. beccariana (5)
R. biasolettiana (1)
R. globiceps (1)
R. rufescens (6)

R. beccariana (9)
R. dura (2)
R. globiceps (2)
R. planctonica (4)

R. beccariana (10)

R. beccariana (9)
R. dura (2)
R. planctonica (1)

R. beccariana (8)

R. beccariana (9)

R. beccariana (8)

R. beccariana (9)

R. beccariana (9)

R. beccariana (9)

R. globiceps (10)
R. haematites (1)
R. planctonica (3)

R. beccariana (1)
R. haematites (1)
R. rufescens (4)
R. viellardi (2)

R. aquatica (1)
R. beccariana (3)
R. dura (1)

R. aquatica (7)
R. beccariana (1)

R. aquatica (1)
R. beccariana (9)

R. aquatica (2)
R. beccariana (7)
R. haematites (1)

R. aquatica (4)
R. beccariana (5)

R. aquatica (4)
R. beccariana (2)
R. dura (1)
R. rufescens (3)

R. beccariana (10)

R. beccariana (10)

R. aquatica (4)
R. beccariana (3)
R. biasolettiana (1)
R. minutula (2)

R. beccariana (10)

R. beccariana (8)
R. biasolettiana (1)
R. rufescens (3)

R. haematites (8)
R. rufescens (4)

In the majority of cases trichomes from one colony keyed-down to several of the established species and because of this it is extremely difficult to assign a specific name. The difference in names assigned, using Geitler and 'IDENTIFY' are to be expected when using such different techniques (see also Section 7.7). In practice it is imperative that workers state which technique and/or flora they have used to identify species.

The results obtained using 'IDENTIFY' (with the complete set of characters) to key down field material can be arranged in seven groups:

(1) Colonies with Rivularia aquatica as the dominant name
(2) Colonies with R. beccariana as the dominant name
(3) Colonies with R. biasolettiana as the dominant name
(4) Colonies with R. dura as the dominant name
(5) Colonies with R. haematites as the dominant name
(6) Colonies with R. planctonica as the dominant name
(7) Colonies with no obvious dominant name

Most of the colonies collected were included under categories (1), (2) and (7). The majority (18 out of 44 colonies) had R. beccariana as the dominant name, although the colonies themselves were collected from a wide range of areas. 9 of the 44 colonies had R. aquatica as the dominant name, of these, 5 were associated with tufa deposits and 3 were from pond sites. 11 colonies did not have a dominant name. From the point of view of future workers, the material from Malham (SD 912656) was the most uniform. Material from this area

was also relatively free of the trichomes of Schizothrix, which had proved impossible to eliminate in culture. Colonies from Malham have also been observed to form new colonies in culture (Section 3.4).

Coding the three strains of Calothrix used for experimental purposes (Chapter 4), and naming them using 'IDENTIFY' produced the following results:

(1) C. brevissima keyed down to C. brevissima (17 matches, 3 mismatches)

(2) C. scopulorum keyed down to C. consociata (13 matches, 5 mismatches)

(3) C. viguieri keyed down to C. clavata (17 matches, 4 mismatches)

For interest, one new strain and 3 new varieties of Calothrix, described by Vasishta (1962) were keyed down against the strains given in Appendix III. The results are as follows:

(1) C. intracellularis sp. nov. keyed down to C. epiphytica (11 matches, 3 mismatches)

(2) C. epiphytica var. major keyed down to C. stagnalis (14 matches, 4 mismatches)

(3) C. gloeocola var. tenuis keyed down to C. stagnalis (14 matches, 4 mismatches)

(4) C. scytonemicola var. major keyed down to C. epiphytica (12 matches, 3 mismatches)

As mentioned above, the different names obtained are partly a result of using a different technique for identification. C. scopulorum and C. consociata are however among those strains described as closely

resembling each other (Table 5.1) and it seems possible that after intensive study, the names of members of this genus may be revised.

5.42 Answering data queries

The data (Appendix II) were used with a computer program 'QUESTION', a copy of which was given in Section 2.11. The program was designed to list species of Calothrix and Rivularia, which possessed certain characters or combinations of characters. Some examples of how to set the questions are given with the copy of the program. 'QUESTION' has been used to summarise rapidly, certain aspects of the morphology of Calothrix and Rivularia. As little information concerning the hair was obtained from laboratory cultures (Section 3.4) the program was used in particular, to study aspects of morphology which might be associated with the presence or absence of a hair.

5.421 Tapering and the hair

The presence or absence of hairs in the 78 species of Calothrix and 22 species of Rivularia (Appendix II) is shown in Table 5.4. Of the 78 species of Calothrix, 21 were described as having a long hair and 6 as having a short hair. 21 species of Rivularia were reported to possess a hair, the hair being long in 12 species and short in 1 species.

The majority of species studied possessed trichomes with a gradual taper i.e. 67 species of Calothrix and 19 species of Rivularia. 5 species of Calothrix and 1 of Rivularia had trichomes which tapered abruptly, while 6 species of Calothrix were reported to have trichomes with a very slight taper. The hair length in relation to the taper of each species is shown in Table 5.5.

long	short	present	absent	not recorded
Calothrix bharadwaja	C. flauhaultii	C. adscendens	C. aeruginosa	C. atricha
C. braunii	C. fusco-violacea	C. aerúginea	C. antarctica	C. columbiana
C. calida	C. gardneri	C. africana	C. bossei	C. consociata
C. castellii	C. parva	C. clavata	C. breviarticulata	C. fulleborni
C. contarenii	C. pulvinata	C. clavatoides	C. brevissima	C. ghosei
C. crustacea	C. simulans	C. confervicola	C. conica	C. kuntzei
C. doliochomeres	C. rufescens	C. cylindrica	C. desertica	C. minuscula
C. donnellii		C. epiphytica	C. elenkinii	C. robusta
C. fusca		C. evanescens	C. gelatinosa	C. violacea
C. geitonos		C. fasciculata	C. gracilis	C. wembaerensis
C. gloeocola		C. floccosa	C. intricata	R. peguana
C. goetzei		C. javanica	C. marchica	
C. juliana		C. linearis	C. parva	
C. kawraiskyi		C. membranacea	C. pilosa	
C. kossinskajae		C. minima	C. submarchica	
C. parasitica		C. parietina	C. viguieri	
C. sandviencis		C. prolifera		
C. santapaui		C. ramenskii		
C. simplex		C. scopulorum		
C. sphaerospora		C. scytonemicola		
C. thermalis		C. stagnalis		
Rivularia aquatica		C. stellaris		
R. beccariana		C. tenella		
R. biasolettiana		C. vivipara		
R. bornetiana		C. weberi		
R. dura		R. atra		
R. globiceps		R. australis		
R. haematites		R. borealis		
R. mamillata		R. bullata		
R. manginii		R. hansgirgi		
R. nitida		R. mesenterica		
R. planctonica		R. minutula		
R. polyotis		R. viellardi		

gradual taper

* Calothrix adscendens
* C. africana
C. antarctica
C. atricha
*** C. bharadwaja
C. bossei
*** C. braunii
C. breviarticulata
*** C. calida
*** C. castellii
* C. clavata
* C. clavatoides
C. columbiana
C. consociata
*** C. contarenii
*** C. crustacea
* C. cylindrica
*** C. donnellii
C. elenkinii
* C. epiphytica
* C. evanescens
* C. fasciculata
** C. flahaultii
* C. floccosa
C. fulleborni
*** C. fusca
** C. fusco-violacea
*** C. geitonos

con.

C. gelatinosa
*** C. gloeocola
C. gracilis
C. intricata
* C. javanica
*** C. juliana
*** C. kawraiskyi
*** C. kossinskajae
C. kuntzei
* C. linearis
C. marchica
* C. membranacea
* C. minima
C. minuscula
*** C. parasitica
* C. parietina
C. parietina var. africana
** C. parva
* C. prolifera
** C. pulvinata
* C. ramenskii
*** C. sandviencis
*** C. santapaui
* C. scopulorum
* C. syctonemicola
*** C. simplex
** C. simulans
*** C. sphaerospora

con.

* C. stagnalis
* C. stellaris
C. submarchica
* C. tenella
*** C. thermalis
C. viguieri
C. violacea
* C. vivipara
C. weberi
C. wembaerensis
* Rivularia atra
*** R. aquatica
*** R. beccariana
*** R. biasolettiana
R. borealis
*** R. bornetiana
* R. bullata
*** R. globiceps
*** R. haematites
*** R. mamillata
*** R. manginii
* R. minutula
*** R. nitida
R. peguana
*** R. planctonica
*** R. polyotis
** R. rufescens
* R. viellardi

abrupt taper

* C. aeruginea
* C. confervicola
** C. gardneri
*** C. goetzei
C. robusta
* R. mesenteric

* hair present
** short hair
*** long hair

The distribution of hairs in relation to tapering may be summarised as follows:

	Calothrix		Rivularia	
	+ hair	- hair	+ hair	- hair
gradual taper	48	19	18	1
abrupt taper	4	1	1	0
slight taper	0	6	0	0

These figures indicate that in general hairs are found more frequently in Rivularia than Calothrix. One interesting point however is the high percentage of species of Calothrix which have a trichome with an abrupt taper and also possess a hair.

5.422 Physiognomic form and the hair

Members of the genus Rivularia frequently possess macroscopic colonies which are spherical or hemispherical in shape. All of the species described in the literature, with the exception of R. planctonica, possess a hair, this being near the outer edge of the colony. As several members of the genus Calothrix have colonies of a similar form, although smaller, the presence of hairs in these species was studied. The results are summarised below, lists including specific names being given in Table 5.6.

	Spherical or hemispherical colony		Other growth form	
	Calothrix	Rivularia	Calothrix	Rivularia
number having each growth form	12	17	37	0
% with hairs	75%	100%	57%	-
% with long hairs	58%	71%	22%	-

Table 5.6 Physiognomic form and the hair in Calothrix and Rivularia

hemispherical or spherical colony

Calothrix	Rivularia
*** C. braunii	* R. atra
*** C. contarenii	*** R. aquatica
*** C. crustacea	* R. australis
C. desertica	*** R. beccariana
*** C. doliochomeres	*** R. biasolettiana
*** C. donnellii	* R. borealis
* C. fasciculata	*** R. bornetiana
*** C. geitonos	*** R. dura
*** C. kossinskajae	*** R. globiceps
C. pilosa	*** R. haematiles
* C. scopulorum	*** R. mamillata
C. wembaerensis	*** R. manginii
	* R. minutula
	*** R. nitida
	*** R. planctonica
	*** R. polyotis
	** R. rufescens

other growth forms

Calothrix	Rivularia
* C. aeruginea	-
C. atricha	
*** C. bharadwaja	
C. bossei	
*** C. calida	
*** C. castellii	
* C. clavatoides	
C. columbiana	
* C. confervicola	
C. consociata	
* C. cylindrica	
C. elenkinii	
** C. flahaultii	
* C. floccosa	
C. fölleborni	
** C. fusco-violacea	
C. gelatinosa	
C. ghosei	
*** C. goetzei	
C. gracilis	
C. intricata	
*** C. juliana	
*** C. kawraiskyi	
C. kuntzei	
* C. membranacea	

con.

Calothrix	Rivularia
* C. minima	
C. minuscula	
* C. parietina	
C. parietina var. africana	
C. prolifera	
** C. pulvinata	
C. robusta	
*** C. simplex	
*** C. thermalis	
C. viguieri	
C. violacea	
C. vivipara	

* hair present
** short hair
*** long hair

The results indicated that a relatively greater percentage of species of Calothrix with hairs have a spherical or hemispherical growth form that is similar to that found in the genus Rivularia. This may possibly be associated with a polarity within the colony which influences the development of apical hairs e.g. gradients of light, concentrations of nutrients and gases. At present however there is no published work describing such gradients.

5.423 Swollen base and the hair

The results discussed in Section 3.21 seemed to indicate that in a high proportion of the species of Calothrix studied in the laboratory, tapering was due to relatively greater increases in the basal width of the trichome i.e. that they had a swollen base. Few of the species in the laboratory possessed hairs and so the distribution of hairs among species with and without swollen bases in nature was studied,(Table 5.7). 35 species of Calothrix and 1 species of Rivularia described in Appendix II were reported to have swollen bases. Of these, the one species of Rivularia and 25 (72%) of the Calothrix species possessed hairs. However a similar number of species of Calothrix 24 (63%), also possessed hairs, but had no swollen base and there appears to be no relationship between the presence of hairs and a swollen trichome base. The lack of hairs in culture seems likely to be due in part to the culture conditions (see also Section 3.4).

5.424 Sheath colour and the hair

The description of C. scopulorum cultured in V_{37}, (Section 3.4)

swollen base and hair in Calothrix and Rivularia

Calothrix	swollen base	Rivularia
+	-	+
C. bossei	* C. adscendens	R. dura
*** C. braunii	* C. aeruginea	
*** C. castellii	C. aeruginosa	
* C. clavata	* C. africana	
* C. clavatoides	C. antarctica	
C. conica	C. atricha	
C. consociata	C. breviarticulata	
* C. contarenii	C. brevissima	
*** C. crustacea	C. columbiana	
* C. cylindrica	* C. confervicola	
C. desertica	*** C. doliochomeres	
C. elenkinii	*** C. donnellii	
* C. fasciculata	* C. epiphytica	
*** C. fusca	* C. evanescens	
* C. fusco-violacea	** C. flahaultii	
** C. gardneri	* C. floccosa	
*** C. gloeocola	C. fülleborni	
C. gracilis	*** C. geitonos	
* C. juliana	C. gelatinosa	
*** C. kawraiskyi	C. ghosei	
*** C. kossinskajae	C. intricata	
C. kuntzei	* C. javanica	
* C. linearis	C. marchica	
C. minuscula	* C. membranacea	
*** C. parasitica	* C. minima	
* C. parietina	** C. pulvinata	
** C. parva	*** C. santapaui	
C. parietina var. africana	* C. scopulorum	
* C. prolifera	* C. scytonemicola	
* C. ramenskii	** C. simulans	
*** C. sandvicencis	*** C. sphaerospora	
*** C. simplex	C. submarchica	
* C. stagnalis	* C. tenella	
* C. stellaris	*** C. thermalis	
C. viguieri	C. violacea	
	* C. vivipara	
	* C. weberi	
	C. wembaerensis	

Rivularia	
-	
* R. atra	
*** R. beccariana	
*** R. biasolettiana	
*** R. globiceps	
*** R. mamillata	
*** R. polyotis	

* hair present
** short hair
*** long hair

was one instance of the formation of hairs in trichomes with a yellow-brown sheath. As the author once observed hairs in culture, reports of these characters occurring together in the literature were studied. 75% of the species of Calothrix with a yellow-brown sheath possessed hairs, while 61% of those with a colourless sheath also possessed hairs.(Table 58) There appears to be a slight tendency for species with yellow-brown sheaths to possess hairs. This may be related to the fact that the hair and yellow-brown sheath appear during the later stages of development of the trichome or that they are influenced by similar environmental factors. At present however there is no evidence concerning the latter point.

5.5 Discussion

An attempt has been made to overcome some of the problems encountered when using the traditional floras to identify Calothrix and Rivularia. An alternative, objective scheme for identifying material has been devised (Section 5.2). This technique was used with each known species, in turn, representing the unknown species. The results show that using the coefficient of resemblance S_{SM} the values for each of the two pairs of species (i) Calothrix atricha and C. scytonemicola and (ii) C. evanescens and C. membranacea, were identical ($S_{SM}=1$). These pairs of species had 14 and 16 identical characters respectively, and 0 characters which were different. Using S_{SK} as the coefficient of resemblance, C. evanescens and C. membranacea were identical i.e. every character which scored other

Table 5.8 Sheath colour and the hair in Calothrix and Rivularia

yellow-brown sheath

Calothrix	Rivularia
* C. africana	*** R. beccariana
C. breviarticulata	*** R. biasolettiana
*** C. calida	*** R. haematites
* C. confervicola	* R. minutula
C. consociata	*** R. polyotis
*** C. crustacea	** R. rufescens
* C. fasciculata	
* C. parietina	
* C. parva	
C. parietina var. africana	
C. pilosa	
** C. pulvinata	
* C. ramenskii	
*** C. santapaui	
* C. scopulorum	
* C. vivipara	

colourless sheath

Calothrix	Calothrix con.	Rivularia
* C. adscendens	*** C. kawraiskyi	* R. borealis
C. aeruginosa	*** C. kossinskajae	*** R. dura
C. atricha	* C. linearis	*** R. globiceps
*** C. bharadwaja	C. marchica	*** R. planctonica
C. bossei	* C. membranacea	
*** C. braunii	* C. minima	
C. brevissima	C. minuscula	
* C. clavata	*** C. parasitica	
* C. clavatoides	*** C. sandviencis	
C. columbiana	* C. scytonemicola	
C. conica	*** C. simplex	
*** C. doliochomeres	** C. simulans	
*** C. donnellii	*** C. sphaerospora	
C. elenkinii	* C. stagnalis	
* C. epiphytica	* C. stellaris	
C. fullehorni	C. submarchica	
*** C. fusca	* C. tenella	
** C. fusco-violacea	C. viguieri	
** C. gardneri	C. violacea	
** C. geitonos	* C. weberi	
C. gelatinosa	C. wembaerensis	
C. ghosei		
*** C. glaeocola		
C. gracilis		
C. intricata		
* C. javanica		
*** C. juliana		

* hair present
** short hair
*** long hair

than 0 or 9 was identical. The nearest neighbours of C. atricha were C. bharadwaja and C. elenkinii and these were not identical when using S_{SK}. Descriptions of these pairs of species, from Geitler (1932) are very similar, except for slight differences in the trichome diameter. When such confusion is possible, it seems preferable to use an objective scheme of identification.

The identification scheme developed has also been used to assign names to 'unknowns'. It was used primarily to name material which was collected from the field (Section 5.412). On the majority of occasions, colonies contained trichomes which keyed-down to several different names and it was not possible to give 'one name' to the colony. This appears to be a reflection of the variation shown by trichomes of one colony in terms of age and possibly response to the environment. The advantage of the scheme which has been devised lies in its objective nature. Although data collection for such a scheme is time consuming initially it can be justified for two reasons. Firstly, that it highlights the inadequecies in the known data and so should promote more rigorous data collecting in the future, preferably in a standard format. Secondly, once the data have been collected it can be readily processed by computer. This has been made use of (Section 5.42) by using the program 'QUESTION' to investigate the relationship between the presence of hairs and four aspects of morphology.

Observations of Calothrix and Rivularia suggested that hairs were present more frequently in Rivularia than Calothrix, and this also seemed to be the case when considering reports from the literature

(Section 5.421). The possession of a hair appears to be related to the development of a spherical or hemispherical growth form and may also be associated with a polarity within such a colony (Section 5.423). There appears to be some tendancy for species of Calothrix and Rivularia, which possess a brown-yellow sheath to possess hairs; however, there does not seem to be any relationship between the possession of hairs and the presence of a swollen trichome base.

6 AN ECOLOGICAL STUDY OF RIVULARIA

6.1 Introduction

The present study was restricted to the genus Rivularia (Section 1.5) and was designed primarily to find possible relationships between water chemistry and the distribution of Rivularia. It was hoped that the results obtained would suggest factors which might possibly affect the development of the hair. The author received a number of personal reports concerning the occurrence of Rivularia, (D.J. Bellamy, K. Benson-Evans, M.E. Bradshaw, B.A. Whitton and P.F. Williams), which helped considerably during this survey.

As mentioned in Section 1.42, West and Fritsch (1927) described the occurrence of members of the Rivulariaceae in upland streams, particularly in limestone regions. As the majority of personal reports (above) were related also to sites in limestone regions, the present study was centred around these areas. The regions from which samples were collected are shown in Fig. 6.1, where they are superimposed on a map showing the distribution of calcareous rocks of Britain.

As samples were collected it became evident that some streams within a particular area contained Rivularia and that others did not. In the majority of ' + Rivularia streams', the colonies formed a conspicuous part of the algal flora, although in some, a few scattered colonies were observed. The locations of these latter streams are given for the sake of interest, but for comparisons of water chemistry, only streams of the former type have been considered to be '+ Rivularia sites! Negative (-) Rivularia sites were streams which (a) drained through or originated from the same limestone outcrop

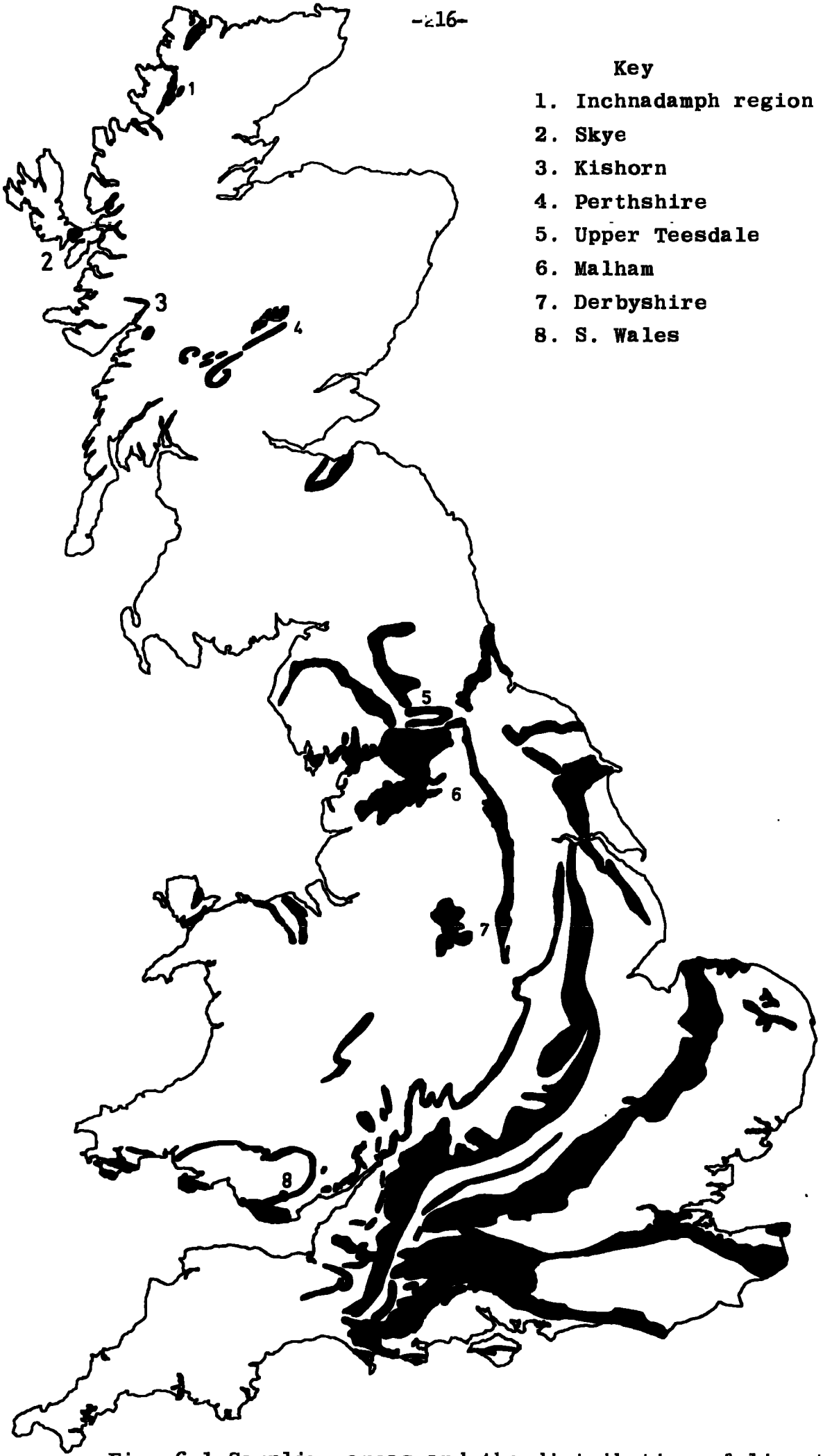


Fig. 6.1 Sampling areas and the distribution of limestone

as the '+ streams' or (b) were in very close proximity to '+ streams' and so were in the same watershed and presumably had similar conditions of temperature and rainfall.

6.2 Site descriptions

6.21 Sampling sites near Inchnadamph, Sutherland

The stream lengths studied in this area were between 91.5-218 m above sea level. They were located in the area to the south west of Quinag and to the east of Loch Assynt, within a small area of 15 km^2. The reaches of the streams which were sampled are shown in Fig. 6.2, where they are superimposed on a map showing the geology of the area (Powell 1970). The area is one of complex geology, Assynt limestone being exposed in several places, particularly in the area to the north east of Inchnadamph. The streams within this region flowed over a range of rock types which would therefore influence their water chemistry. The influence of this on the presence or absence of Rivularia was studied. In some streams there were obvious changes in the abundance of Rivularia downstreams and hence several reaches within one stream were compared.

6.22 Additional Scottish sampling sites

In addition to a detailed study of the Inchnadamph region, a brief survey was made in several separate limestone regions of Scotland. In two of the regions, at Kishorn and on Skye, the limestone outcrops were difficult to locate as they were very narrow. The two other sites sampled were at Knockan (Sutherland) and Blair Atholl (Perthshire). Most of these sites were visited during the early

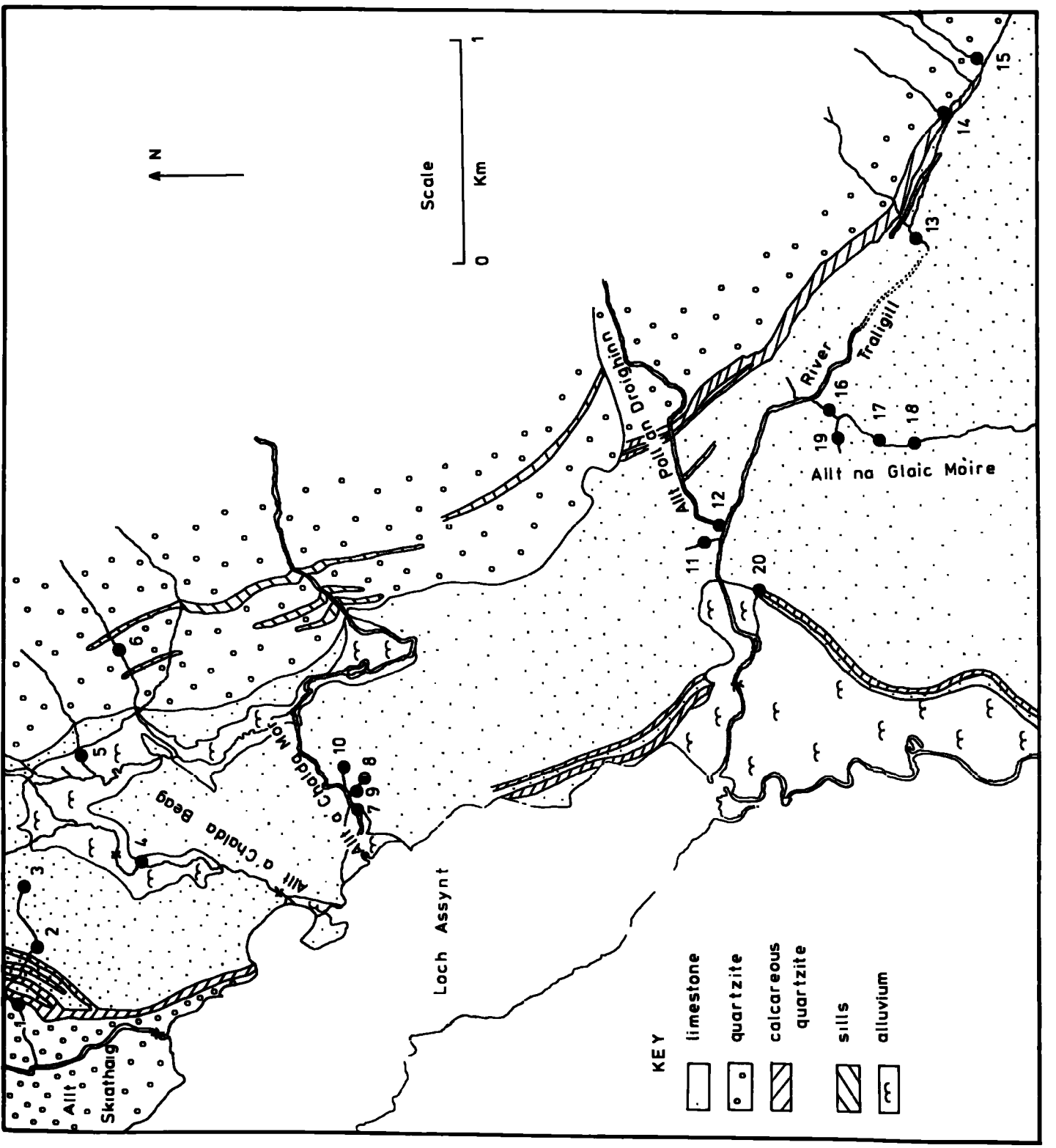


Fig. 6.2 Sampling sites in the Inchnadamph region of N.W. Scotland

part of the survey and were included because they all drained over and/or from limestone; however, they are widely distributed geographically. The abundance of Rivularia decreased noticeably at successive downstream sites in the Blair Atholl stream and several reaches within this one stream were sampled.

6.23 North Pennines and Upper Teesdale

One of the main areas from which Rivularia was collected was Upper Teesdale. This region is famous for its higher plant rarities which were described by Piggott (1956). More recently, Hughes and Whitton (1972) have described the algae of Slapestone Sike, a small stream draining Widdybank Fell.

Manley (1942) emphasised the sub-arctic nature of the environment, although the area is only 840 m above sea level. The stream reaches which were sampled are shown in Fig. 6.3. The geology of the area has been described by Piggott (1956) and by Johnson, Robinson and Hornung (1971). The streams flow over exposed metamorphosed limestone ('sugar limestone'). Rivularia was attached to many of the slabs of this rock and to loose Whin Sill pebbles in the streams.

Rivularia has been collected from several parts of Teesdale by Dr M.E. Bradshaw, viz. from Bowlees quarry, a Whin Sill quarry near Middleton and from Cronkley Fell. Material from the first two of these sites has been coded (Appendix IV), but water analyses were not available.

6.24 Westmorland

The main area of study in Westmorland was a region of wet flushes near Sunbiggin Tarn. The area, described by Holdgate (1955) lies in the north west Pennines at an altitude of 250 m. The area is

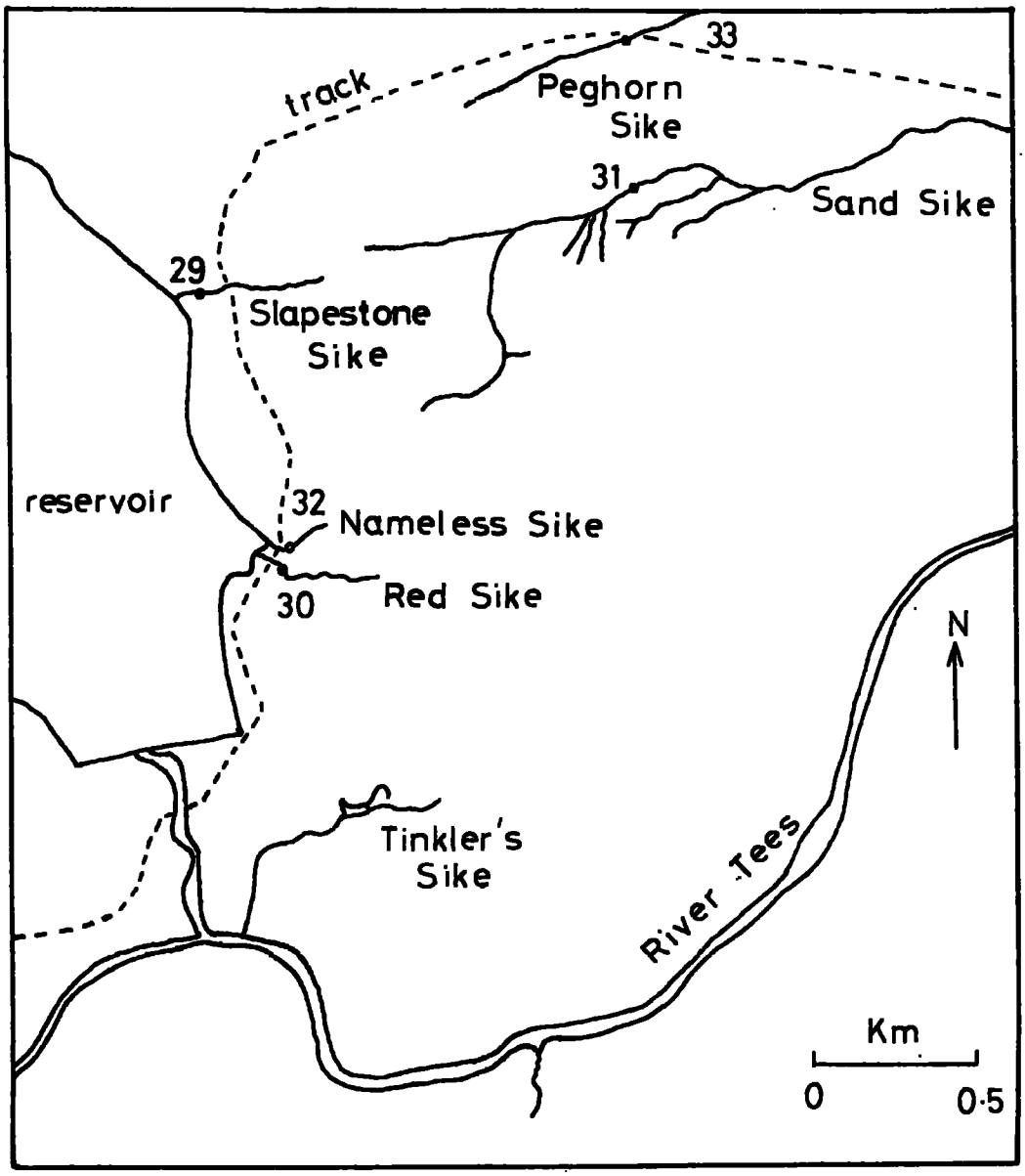


Fig. 6.3 Sampling sites in Upper Teesdale

mainly Carboniferous limestone, with bands of sandstone and drift. The drainage of the area is complex and there are numerous springs and swallow holes. Below the springs, Holdgate described the area as having shallow peat with a high mineral content and stony calcareous clays. He made several references to the wet flushes described by Pearsall (1950) and also to similar areas in Swedish soligenous fens, known as 'Flarks' (Sjors 1948, 1950). A stream at Barras (NY 847122) also contained Rivularia.

6.25 Malham region, West Yorkshire

The geology of Malham Tarn and surrounding regions has been described by O'Connor (1964). It is mainly a large area of exposed Carboniferous limestone, although there are areas where the older rock is exposed. In the north west Fountains Fell rises to a height of 669 m (O'Connor 1964), while in the north east a high limestone escarpment rises to about 457 m. South of this the land drops to an extensive platform around the Tarn (approximately 381 m). Continuing southwards there is a steep drop across the Mid-Craven fault to 185 m at Malham.

Several reaches along Gordale Beck and its tributary streams were studied (Fig. 6.4). The streams drain the eastern side of the Aire-Ribble watershed and flow into the River Aire. One site on the northern shore of Malham Tarn was also sampled.

A few streams in nearby areas were also examined for the presence or absence of Rivularia. A few colonies were found in two streams (i) associated with the tufa deposits in a flush opposite the entrance to Ingleborough cave (SD 756712) and (ii) in Austwick Head stream

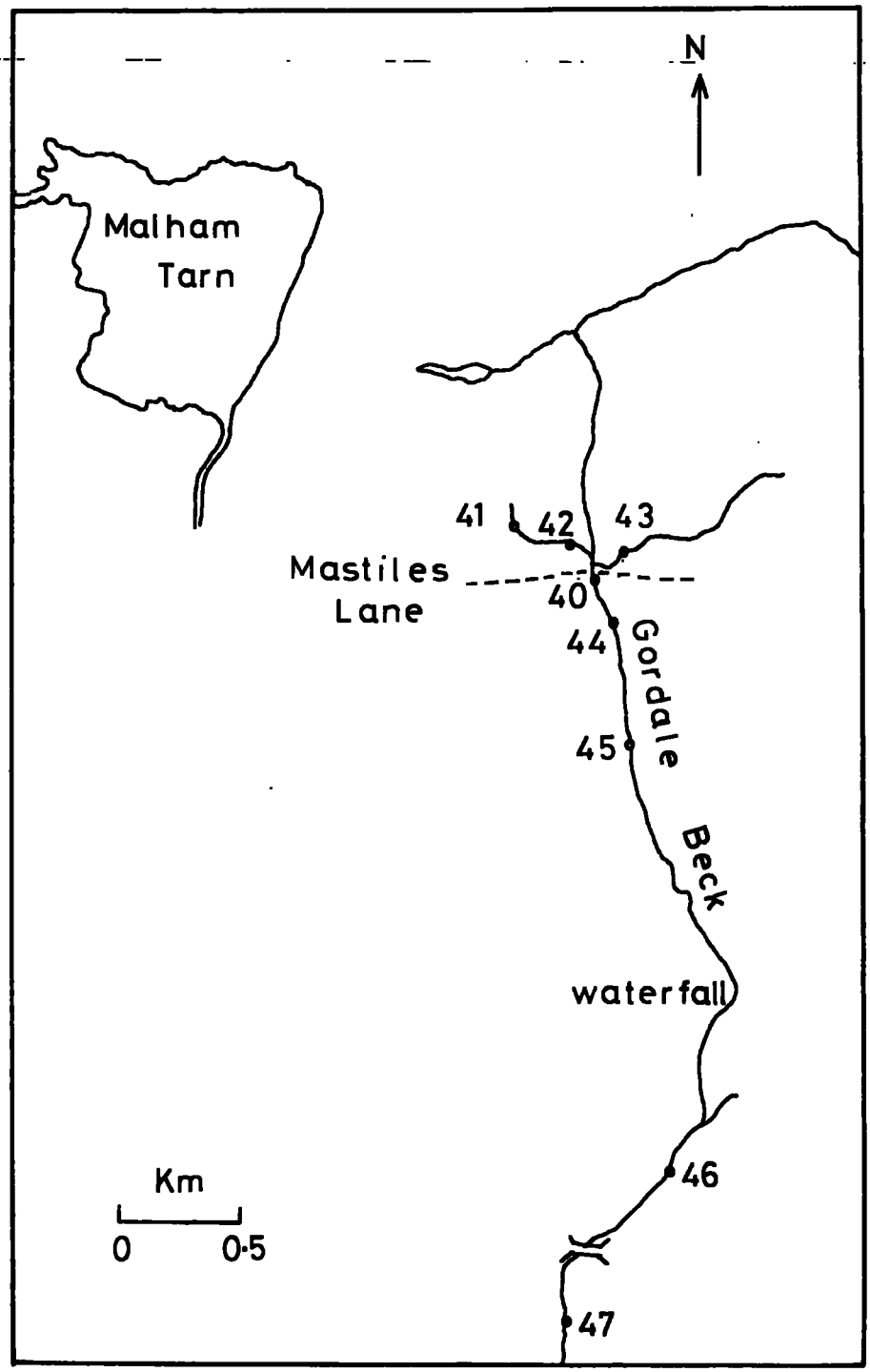


Fig. 6.4 Sampling sites in the Malham region

(SD 776718). Rivularia was not found in the stream emerging in Malham Cove (SD 897641).

6.26 South Wales

A small area of the South Wales limestone was included in this survey. Pringle and Neville George (1948) described the Carboniferous outcrop around the South Wales coalfield. It extends in a narrow strip running east from Kidwelly in Carmarthenshire to near Abergavenny, where it runs south near Pontypool. Behind Cardiff the limestone runs westwards beyond Porthcawl. Several streams associated with the band of limestone behind Cardiff were studied. Only one of the streams, that at Cwm Nofydd, was found to contain Rivularia.

6.3 Water chemistry data

Water samples were collected from the sites listed in Table 6.1. The samples were collected and analysed as described in Section 2.8, the results being given in Table 6.2. Upper and lower limits for each component of water chemistry and the range over which Rivularia occurred, are given in Table 6.3. Changes in the water chemistry within individual streams in which the abundance of Rivularia decreased downstream, are given in Tables 6.4 and 6.5. The levels of each component of water chemistry, from sites with and without Rivularia, were compared using the Mann-Whitney U-test (Section 2.12). Comparisons were made between + and - streams in individual regions and between all '+ Rivularia sites' and all '- Rivularia sites'. In cases where the number of + and - sites was small, comparisons were made by inspecting Table 6.2.

Table 6.1 Sites sampled during *Rivularia* survey

Site number	+ or - *Rivularia*	location	Grid reference	Date
N.W. Scotland				
2	+	Allt Skiathaig	NC 237251	24.9.72
3	+	" "	NC 238256	"
8	+	side flush of Allt a Chalda Mor	NC 246236	"
10	+	" "	NC 247235	"
17	+	Allt na Glaic Moire	NC 267213	25.9.72
18	+	" "	NC 262210	"
19	+	" "	NC 260216	"
20	+	spring near Inchnadamph Hotel	NC 255217	"
1	-	Allt Skiathaig	NC 236250	24.9.72
4	-	Allt a Chalda Beag	NC 242245	"
5	-	stream flowing into Allt nam Breac	NC 247250	"
6	-	" "	NC 252245	"
7	-	Allt a Chalda Mor	NC 245235	"
9	-	side flush of Allt a Chalda Mor	NC 246236	"
11	-	stream flowing into Allt Poll an Droighinn	NC 260221	25.9.72
12	-	Allt Poll an Droighinn	NC 261220	"
13	-	Traligill River	NC 272210	"
14	-	stream flowing into Traligill	NC 279213	"
15	-	" "	NC 277205	"
16	-	Allt na Glaic Moire	NC 267213	"
Other Scottish sites				
25	+	Kishorn, stream	NG 842432	24.8.71
26	+	"	"	"
21	+	Tulach hill, stream	NN 869643	23.9.72
22	+	"	NN 869644	"
23	+	"	NN 869645	"
24	-	Tulach hill, stream	NN 868642	23.9.72
27	-	Skye, Torrin	NG 589205	24.8.71
28	-	stream near Knockan	NC 101199	24.9.72
North Pennines and Upper Teesdale				
29	+	Slapestone Sike	NY 814304	10.9.72
30	+	Red Sike	NY 816294	"
31	+	Sand Sike	NY 828308	"
32	+	Nameless Sike	NY 817296	14.4.72
33	-	Peghorn Sike	NY 828312	10.9.72

Table 6.1 continued

Site number	+ or - Rivularia	location	Grid reference	Date
Westmorland				
34	+	Sunbiggin flush	NY 672077	10.9.72
35	+	"	"	"
36	+	"	"	"
37	-	Tarn Sike	NY 672076	16.4.72
38	-	Rais Beck	NY 648074	10.9.72
Malham region, West Yorkshire				
40	+	Gordale Beck, ford	SD 912656	5.10.72
41	+	Gordale Beck, side flush	SD 909655	"
42	+	"	SD 909656	"
44	+	Gordale Beck	SD 912654	"
45	+	"	SD 912651	"
43	-	side stream flowing into Gordale Beck	SD 913657	5.10.72
46	-	Gordale Beck, below waterfall	SD 914637	"
47	-	Janet's Cove, above waterfall	SD 912663	16.10.71
South Wales				
48	+	Cwm Nofydd	ST 147835	10.9.71
49	-	Coed-y-Bedw	ST 118843	"
50	-	Draethen	ST 205865	"

Table 6.2 Water analyses (concentrations in mg l^{-1})

site number	+ or - Rivularia	OD_{420}	pH_{field}	cond. µmhos	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Cl	Si
North West Scotland																
2	+	0.0067	7.1	290	8.4	0.70	27.0	44.3	0.002	0.007	0.002	0.03	0.03	0.002	20.5	1.20
3	+	0.0025	7.6	320	8.5	0.64	27.5	46.9	0.002	0.014	0.002	0.01	0.03	0.002	21.0	1.55
8	+	0.0010	7.2	335	8.6	0.78	30.0	47.0	0.002	0.016	0.002	0.02	0.07	0.002	22.0	1.15
10	+	0.0050	7.6	295	8.5	0.70	26.0	41.4	0.002	0.009	0.002	0.01	0.07	0.002	22.6	1.20
17	+	0.0110	8.2	255	8.7	1.72	21.0	33.4	0.009	0.016	0.002	0.01	0.03	0.002	22.0	1.20
18	+	0.0050	7.9	255	7.7	0.72	20.9	32.8	0.002	0.003	0.002	0.03	0.03	0.002	20.0	1.30
19	+	0.0070	8.0	220	7.0	0.54	18.6	29.9	0.007	0.006	0.004	0.04	0.03	0.002	18.7	1.10
20	+	0.0015	8.2	300	7.8	0.66	28.5	45.8	0.002	0.006	0.002	0.01	0.03	0.001	19.0	1.50
1	-	0.0055	6.8	285	7.9	0.56	26.0	44.1	0.010	0.016	0.002	0.01	0.03	0.003	22.0	1.15
4	-	0.0087	6.8	137	6.9	0.68	10.1	16.4	0.003	0.010	0.002	0.01	0.03	0.002	15.8	2.65
5	-	0.0273	7.0	54.0	6.9	1.54	1.9	3.0	0.003	0.011	0.002	0.04	0.03	0.002	19.0	2.10
6	-	0.0205	6.7	36.3	6.2	0.80	0.8	1.2	0.022	0.012	0.003	0.05	0.03	0.003	18.5	0.90
7	-	0.0110	7.0	43.0	6.5	0.56	1.4	2.9	0.002	0.011	0.006	0.03	0.03	0.002	18.0	1.60
9	-	0.0067	7.0	73.5	7.9	0.66	3.5	6.3	0.009	0.009	0.002	0.03	0.03	0.002	19.3	3.95
11	-	0.0050	7.2	314	8.5	1.02	28.0	45.5	0.010	0.009	0.004	0.02	0.03	0.002	23.2	1.55
12	-	0.0637	7.4	69.0	7.1	1.56	3.2	5.5	0.010	0.009	0.005	0.14	0.03	0.003	20.5	2.70
13	-	0.0130	7.4	48.0	5.5	-	-	-	0.010	0.009	0.022	0.04	0.11	-	20.0	1.20
14	-	0.0692	7.3	45.0	7.2	1.42	1.1	1.7	0.012	0.018	0.014	0.43	0.24	0.003	19.0	0.90
15	-	0.0105	8.2	280	10.1	2.60	23.0	41.8	0.090	0.016	0.034	0.65	0.16	0.008	22.7	2.70
16	-	0.0045	8.5	260	7.6	0.58	21.0	34.0	0.006	0.016	0.003	0.01	0.03	0.001	19.2	1.20
Other Scottish sites																
25	+	-	-	358	7.0	1.50	23.1	72.5	-	-	-	-	-	-	-	3.62
26	+	-	-	394	7.0	1.00	24.8	53.0	-	-	-	-	-	-	-	1.65
21	+	0.0030	7.4	255	5.3	0.34	1.3	79.0	0.009	0.012	0.002	0.01	0.03	0.010	14.6	3.20
22	+	0.0010	7.7	255	4.3	0.74	1.3	73.5	0.013	0.016	0.002	0.03	0.03	0.005	16.0	3.20
23	+	0.0030	8.4	255	4.8	0.74	1.2	71.9	0.005	0.012	0.002	0.03	0.03	0.004	15.0	3.00
27	-	-	-	314	7.0	1.50	11.40	64.1	-	-	-	-	-	-	-	3.85
24	-	0.0045	8.1	235	4.6	0.72	1.23	66.0	0.010	0.012	0.002	0.01	0.03	0.002	15.2	3.45
28	-	0.0042	7.2	265	7.7	0.82	22.00	36.3	0.008	0.012	0.002	0.01	0.03	0.003	21.5	1.30

Table 6.2 continued

site number	+ or - Rivularia	OD_{420}	pH_{field}	cond. µmhos	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Cl	Si
North Pennines and Upper Teesdale																
29	+	0.0080	8.3	270	3.0	0.48	1.12	64.8	0.021	0.009	0.032	0.09	< 0.03	0.002	12.3	1.90
30	+	0.0220	8.5	274	2.9	0.26	0.88	62.8	0.003	< 0.002	0.004	0.02	< 0.03	0.026	13.5	2.05
31	+	0.0120	8.3	172	3.9	1.08	2.71	62.0	0.010	0.007	0.011	0.06	< 0.03	< 0.001	13.4	6.60
32	+	-	-	-	2.3	0.27	1.02	46.7	0.005	< 0.002	0.010	< 0.01	0.07	0.006	-	-
33	-	0.0190	8.1	270	3.5	0.94	2.10	61.8	0.023	0.014	0.061	0.05	< 0.03	0.002	13.4	2.35
Westmorland																
34	+	0.0150	7.9	363	4.6	1.50	3.30	85.0	0.082	0.028	0.032	0.04	< 0.03	< 0.001	15.4	4.90
35	+	0.0020	8.0	328	4.0	0.90	9.20	74.3	0.045	0.009	0.011	< 0.01	< 0.03	< 0.001	15.7	5.35
36	+	0.0040	8.0	392	4.2	0.80	7.80	81.6	0.031	0.007	0.010	< 0.01	< 0.03	< 0.001	15.7	5.50
37	-	-	-	-	4.1	0.67	3.60	54.5	0.009	0.005	0.014	0.09	0.13	0.002	-	-
38	-	0.0050	8.1	265	4.0	0.74	5.95	76.8	0.015	0.021	0.016	< 0.01	< 0.03	< 0.001	15.7	4.15
Malham region, West Yorkshire																
40	+	-	8.3	-	3.5	0.56	1.53	86.0	< 0.002	0.009	0.003	0.01	< 0.03	0.002	-	1.30
41	+	-	8.5	-	3.7	1.14	2.02	75.6	0.007	0.011	0.003	0.02	< 0.03	0.002	-	0.80
42	+	-	8.4	-	3.5	0.52	2.03	73.9	0.007	0.011	0.005	0.02	< 0.03	0.002	-	0.55
44	+	-	8.4	-	3.6	0.84	2.10	64.5	0.006	0.012	< 0.002	0.01	< 0.03	0.002	-	0.70
45	+	-	-	-	4.5	1.14	0.93	57.1	0.008	0.015	0.006	0.02	< 0.03	0.001	-	0.35
43	-	-	8.2	-	3.8	1.04	2.00	71.6	0.009	0.016	0.004	< 0.01	< 0.03	0.003	-	0.70
46	-	-	-	187	4.5	0.24	0.92	40.0	-	-	-	-	-	-	13.5	0.92
47	-	-	-	304	4.7	0.58	-	69.0	-	-	-	-	-	-	15.0	1.05
South Wales																
48	+	-	-	-	11.8	0.75	37.00	71.1	-	-	-	-	-	-	-	-
49	-	-	-	-	12.0	1.75	17.00	67.0	-	-	-	-	-	-	-	-
50	-	-	-	-	8.5	1.50	21.80	76.1	-	-	-	-	-	-	-	-

Table 6.3 Upper and lower limits for each component of water chemistry and for the occurrence of Rivularia (concentrations in mg l^{-1})

	Water chemistry limits		Rivularia limits	
	upper	lower	upper	lower
OD_{420}	0.069	0.001	0.022	0.001
pH_{field}	8.5	6.7	8.5	7.1
$cond_{(\mu mhos)}$	394	36.3	394	172
Na	12.0	2.3	11.8	2.3
K	2.94	0.26	1.72	0.26
Mg	37.00	0.88	37.0	0.88
Ca	86.0	1.2	86.0	29.9
Zn	0.082	< 0.002	0.082	< 0.002
Cu	0.028	< 0.002	0.028	< 0.002
Mn	0.061	< 0.002	0.032	< 0.002
Fe	0.65	< 0.01	0.19	< 0.01
Al	0.24	< 0.03	0.07	< 0.03
Pb	0.026	< 0.001	0.026	< 0.001
Cl	23.2	12.3	22.6	12.3
Si	6.60	0.35	6.60	0.35

Table 6.4 Change in water chemistry of two streams near Inchnadamph (concentrations in mg l^{-1})

	tributary of Allt Skiathaig			Allt na Glaic Moire			
reach	3	2	1	18	17	19	16
+ Rivularia	+	+	-	+	+	+	-
OD_{420}	0.0025	0.0067	0.0055	0.0050	0.0110	0.0070	0.0045
pH	7.6	7.1	6.8	7.9	8.2	8.0	8.5
conductivity (μmhos)	320	290	285	255	255	220	260
Na	8.5	8.4	7.9	7.7	8.7	7.0	7.6
K	0.64	0.70	0.56	0.72	1.72	0.54	0.58
Mg	27.5	27.0	26.0	20.9	21.0	18.6	21.0
Ca	46.9	44.3	44.1	32.8	33.4	29.9	34.0
Zn	0.002	0.002	0.010	0.002	0.009	0.007	0.006
Cu	0.014	0.007	0.016	0.003	0.016	0.006	0.016
Mn	0.002	0.002	0.002	0.002	0.002	0.004	0.003
Fe	0.01	0.03	0.01	0.03	0.01	0.04	0.01
Al	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Pb	0.002	0.002	0.003	0.002	0.002	0.002	0.001
Cl	21.0	20.5	22.0	20.0	22.0	18.7	19.2
Si	1.55	1.20	1.15	1.30	1.20	1.10	1.20

Table 6.5 Change in water chemistry in streams near Blair Atholl and Malham (concentrations in mg l^{-1})

site	Tulach hill stream (Blair Atholl)				Gordale Beck (Malham)					
reach	21	22	23	24	41	42	40	44	45	46
± Rivularia	+	+	+	-	+	+	+	+	+	-
OD_{420}	0.0030	0.0010	0.0030	0.0045						
pH	7.4	7.7	8.4	8.1	8.5	8.4	8.3	8.4	-	-
conductivity (μmhos)	255	255	255	235						
Na	5.3	4.3	4.8	4.6	3.7	3.5	3.5	3.6	4.5	4.5
K	0.34	0.74	0.74	0.72	1.14	0.52	0.56	0.84	1.14	0.24
Mg	1.3	1.3	1.2	1.23	2.02	2.03	1.53	2.10	0.93	0.92
Ca	79.0	73.5	71.9	66.0	75.6	73.9	86.0	64.5	57.1	40.0
Zn	0.009	0.013	0.005	0.010	0.007	0.007	< 0.002	0.006	0.008	-
Cu	0.012	0.016	0.012	0.012	0.011	0.011	0.009	0.012	0.015	-
Mn	0.002	0.002	0.002	0.002	0.003	0.005	0.003	<0.002	0.006	-
Fe	0.01	0.03	0.03	0.01	0.02	0.02	0.01	0.01	0.02	-
Al	0.03	0.03	0.03	0.03	< 0.03	<0.03	< 0.03	< 0.03	<0.03	-
Pb	0.010	0.005	0.004	0.002	0.002	0.002	0.002	0.002	0.001	-
Cl	14.6	16.0	15.0	15.2	-	-	-	-	-	13.5
Si	3.20	3.20	3.00	3.45	0.80	0.55	1.30	0.70	0.35	0.92

6.31 Comparison of water chemistries from + and - Rivularia sites

The results given below indicate the cases in which the probability of the null hypothesis being true (i.e. that there was no significant difference in the water chemistry between + and - sites), was ≤ 0.1.

Inchnadamph region

OD	lower in sites with Rivularia	($p \leq 0.01$)
Mg^{420}	higher " "	($p \leq 0.01$)
Ca	higher " "	($p \leq 0.025$)
Zn	lower " "	($p \leq 0.01$)
Mn	lower " "	($p \leq 0.025$)
Fe	lower " "	($p \leq 0.05$)
Pb	lower " "	($p \leq 0.05$)

Other Scottish sites

OD_{420}	lower in sites with Rivularia	($p \leq 0.10$)
Ca	higher in sites with Rivularia	($p \leq 0.07$)
Pb	higher " "	($p \leq 0.10$)

North Pennines and Upper Teesdale

Inspection of Table 6.2 suggested the following tendancies:

pH	lower in sites with Rivularia
Zn	slightly lower in sites with Rivularia
Cu	lower in sites with Rivularia
Mn	lower " "

Westmorland

Inspection of Table 6.2 suggested the following tendencies:

Conductivity higher in sites with Rivularia

K higher " "

Zn higher " "

Malham region, West Yorkshire

pH higher in sites with Rivularia (inspection Table 6.2)

Na lower in sites with Rivularia ($p \leq 0.04$)

Mg lower " " ($p \leq 0.07$)

Zn slightly lower in sites with Rivularia (inspection of Table 6.2)

Cu " " " " "

Fe " " " " "

Comparison of all + and - sites

OD_{420} lower at sites with Rivularia ($p \leq 0.025$)

pH higher at " " ($p \leq 0.05$)

conductivity higher at sites with Rivularia ($p \leq 0.025$)

Na lower at sites with Rivularia ($p \leq 0.057$)

Ca higher " " ($p \leq 0.005$)

Zn higher " " ($p \leq 0.039$)

Cu lower " " ($p \leq 0.081$)

Fe lower " " ($p \leq 0.097$)

Al lower " " ($p \leq 0.015$)

6.4 Distribution of Rivularia in terms of substratum and water depth

Although Rivularia was found in streams which were associated with limestone bedrock, colonies were not restricted in their

distribution to limestone, as a substratum. In the majority of streams in which Rivularia occurred, limestone pebbles and boulders formed the main substratum e.g. in Gordale Beck. In regions of Upper Teesdale however, colonies were attached to Whin Sill blocks as well as the slabs of metamorphosed limestone. In some streams near Inchnadamph colonies were found attached to quartzite pebbles.

Although not directly related to the present survey of streams, it is interesting to note that in a lowland pond in County Durham, described by Hudson, Crompton and Whitton (1971), Rivularia (incorrectly termed Gloeotrichia in the paper), has been found attached to wood and polythene. The site is of particular interest, being the only example of a'karst' or subsidence lake described for Magnesian Limestone, in Britain (Wheeler and Whitton 1971).

At several of the sites where springs emerged, Rivularia was associated with tufa deposits e.g. Gordale Beck (site 42), Tulach hill stream (site 21) and Sunbiggin (sites 34-36).

All of the sites were visited when the water level of the streams was low. Colonies were found above the water level, but the best growth were submerged. In general the colonies occurred at depths of 10-50 mm, however in Gordale Beck samples were found at 250 mm depth.

6.5 Discussion

During a survey of upland limestone regions in Britain, it has been found that although not widespread, Rivularia is abundant and occasionally dominant in certain streams. The most well documented aspect of the distribution of the genus, i.e. its abundance in

mountain streams in limestone districts (Fritsch 1945, Fritsch and West 1927, Hughes and Whitton 1972), has been confirmed. The location of Rivularia sites on the basis of geological information illustrates the usefulness of such a generalisation (see below).

A consideration of the data available concerning water chemistry (a) on the basis of individual streams (b) on a regional basis and (c) overall, provided the following generalisations. Rivularia tended to occur at sites with relatively low OD_{420} and relatively high pH, although the latter was not obvious from data concerning individual streams. Colonies were found over the complete range of Na levels which were sampled. In general, neighbouring streams both with and without Rivularia had similar levels of Na. Compared to other areas, Na levels in the Teesdale region were low. In the majority of areas, K levels were similar in both + and - Rivularia streams. At Sunbiggin however, K levels in the + Rivularia streams were relatively high, and in South Wales the levels in both the + and - Rivularia streams were high. In contrast, Mg levels at sites with Rivularia were generally high, the exceptions being at Teesdale and Malham, where both + and - Rivularia streams had relatively low levels of Mg.

In areas where there were a variety of rock types (such as Inchnadamph) some of which were calcareous and others which were not, all of the + Rivularia sites had relatively high levels of Ca. In areas where both + and - Rivularia streams drained through limestone, Ca levels were all fairly high. The Ca levels from

individual streams decreased at successive 'downstream' reaches, this feature probably reflecting changes in the source of water, and the inflow of water which had drained through the surrounding land. Exceptions to these generalisations were found in two streams with fairly high Ca levels, but no Rivularia (sites 11 and 15, Inchnadamph).

In very general terms the levels of microelements were lower in + Rivularia streams that in neighbouring - Rivularia streams. An overall comparison suggested that Rivularia occurred at relatively high levels of Zn, however considering the results from individual streams, this tendency was only evident at Sunbiggin. In other areas (Inchnadamph, Teesdale and Malham) the Zn levels were lower in + Rivularia streams.

Cu and Mn levels were both relatively low in streams with Rivularia however, . . . Mn and Cu was relatively high at a + Rivularia site at Sunbiggin and Mn was higher at one of the + sites at Teesdale. Fe levels were slightly lower in streams with Rivularia, however the highest Fe level recorded during the survey was from a site with Rivularia was site 29, Teesdale.

Considering the range of values obtained for Al, Pb, Cl and Si (Table 6.3), Rivularia was found at relatively low levels of Al and over the complete range of concentrations of the other ions. Compared to neighbouring non-Rivularia sites, Pb was relatively high at some sites in Teesdale and Scotland and Si was relatively high in one + Rivularia site in Teesdale and one in Westmorland.

In all of the streams studied, Rivularia was more abundant near to at least one of the sources of the stream. However, where there were several sources, it was sometimes absent from some side flushes e.g. site 9, Inchnadamph and side streams flowing into Slapestone Sike (Hughes and Whitton 1972). In the case of the Allt Skiathaig, the lower parts of the stream flowed through peat and then into a larger, more rapidly flowing stream. The lower reaches of both Tulach hill stream and Gordale Beck flowed through pasture and in the former, the stream probably received run-off from a farm. It seems probable that the source of water is likely to be of considerable importance in determining the presence or absence of Rivularia. To reflect such changes in water chemistry the anions should be analysed as well, however this was not possible during the present study.

A further generalisation concerning the distribution of Rivularia is that it tends to occur in relatively small streams, which are usually fairly shallow and do not dry out completely in dry weather. The majority of colonies occurred in water which was $\leq$ 50 mm deep, however the growths observed in Gordale Beck were present in water up to 250 mm deep. In the few pond sites known to the author, colonies were attached to rocks or pebbles close to the shore and were submerged. In the two Scottish lochs (NC 096181 and NC 105178) and Malham Tarn (SD 897672) there was slight wave action in areas where colonies were found and water drained away from the sites by outflow streams. At Croft (NZ 102108), water flowed into

the pond from an underground source (Hudson, Crompton and Whitton 1971) and therefore in all four situations, the water was not static. Rivularia was never found in large streams with fast flow rates.

In summary, Rivularia, seems to be the dominant alga of certain upland streams in limestone regions. A comparison of the areas from which Rivularia has been recorded with a list of 20 locations of higher plants, known to have a discontinuous distribution (Piggott and Walters 1954) is given in Table 6.6. It seems possible, as suggested by Hughes and Whitton (1972), that Rivularia was once more widespread.

The distribution of Rivularia, although linked to water chemistry seems to be dependent on the source of water as well. Water likely to contain fertilisers may be a factor likely to restrict the distribution. The best covers of Rivularia seen during this survey were those in Gordale Beck and in Red Sike, Teesdale. It is suggested that these areas may be useful sources of material for future research and for more detailed ecological studies within individual streams.

Table 6.6 Comparison of the distribution of Rivularia with 20 localities of plant communities known to have a discontinuous distribution - (+ indicates presence of Rivularia)

Presence of Rivularia	Location
+	Serpentine of the Lizard peninsula, Cornwall
	Devonian limestone, Berry Head, South Devon
	Chalk, Beer Head, South Devon
	Carboniferous limestone, Braen Down and Mendip Hills, North Somerset
	Carboniferous limestone, Avon Gorge, Bristol
	Chalk, Isle of Wight, Hampshire and Dorset
	Chalk, Surrey and Sussex
	Chalk, Kent
	Chalk, East Anglia and Herts
	Carboniferous limestone, Gower Peninsula, South Wales
	Carboniferous limestone, North Wales, Anglesey
+	Carboniferous limestone, Derbyshire
	Basic igneous, Crag Breiddon, Montgomery and Stanner Rocks,Radnor
+	Carboniferous limestone, Ingleborough and Craven district, etc. Yorkshire and Westmorland
+	Carboniferous limestone (locally metamorphosed) Teesdale
	Carboniferous limestone, Humphrey Head
	Basic igneous, North Queensferry and Arthur's Seat, Edinburgh
+	Dalradian limestone, Rannoch and Perthshire
+	Durness limestone, Inchnadamph, Cnochan and Bettyhill,Sutherland
+	Carboniferous limestone, Burren, Co. Clare, Ireland.

Record for (1) from Bold (1966), record for (12) pers. comm. Prof. Chesters record for (20) pers. comm. M. Potts.

7 DISCUSSION

7.1 Introductory comments

The results obtained during this thesis have illustrated the difficulties likely to be encountered when using morphological characters for identification purposes. During the present work, tapering (Section 1.31) and hair formation (Section 1.32) in two genera, Calothrix and Rivularia, were studied. Both characters showed considerable variation and prior to the present study have been described only in qualitative or semi-quantitative terms. As a prerequisite to further studies the author has suggested ways of describing the taper (Section 3.2) and the hair (Section 3.3) quantitatively. These methods were used to study the effect of certain factors on tapering (Section 4.2 and 4.3) and on hair formation (Section 3.4). Most of the experimental work was concerned with the effect of combined nitrogen on tapering; however as the production of heterocysts and the nitrogen fixing ability were also likely to change, these were studied as well. The results of this part of the work have several implications to the taxonomy of the Rivulariaceae (Section 7.5). The problems associated with the need to assign a name in order to pass on information, led the author to devise an objective method of naming members of the genera Calothrix and Rivularia (Chapter 5). The results of an ecological survey which was designed to find possible links between the distribution of Rivularia and water chemistry (Section 6.3) are discussed in Section 7.8. The significance of the gross morphology to the distribution of the genus is also considered (Section 7.8).

7.2 Tapering

Tapering has been described in quantitative terms for the first time (Section 3.21). Four indices of tapering, designated T_1, T_2, T_3 and T_4 (p. 73,80) have been derived from the simple tapered shape of a cone. The simplest shape is that of a cone in which the rate of change in diameter is uniform per unit length (Fig. 3.1, p. 72). Associated with this shape are the three parameters: maximum width, minimum width and length. Values for T_1 or T_2 are calculated using the first two of these parameters, while T_3 or T_4 are calculated using all three parameters.

These indices of tapering have been used to quantify differences in tapering due to the presence of combined nitrogen (Sections 4.2 and 4.3). Statistical tests have shown that there is no significant difference in the values obtained for T_1 and T_2 from cultures of C. viguieri grown in - N medium for up to 32 days. Values obtained for T_3 and T_4, for the same culture, are significantly different. This difference was brought about by lower values of T_3 and T_4 during the later stages of growth and this in turn reflects growth in length of the trichomes.

A different situation existed for C. scopulorum grown in - N medium for 31 days. The extreme values obtained for T_1 and T_2 are significantly different, while the extreme values of both T_3 and T_4 are not significantly different. This implied that there were changes in the trichome diameter as the culture aged but that the rate of change of diameter was fairly constant.

Having examined the change in the values obtained for the

indices of tapering during growth in - N medium, a comparison was made with cultures grown in + N medium. The four 'T' values obtained for C. viguieri at certain stages of growth in - N and + N were compared. The values obtained for T_1 showed a significant difference between - N and + N cultures after 12-18 days growth, and the values obtained for T_2 showed a significant difference between - N and + N cultures after 18 days. The values obtained for T_3 from the two cultures did not differ significantly until after 32 days, while T_4 values were not significantly different.

Cultures of C. scopulorum from - N and + N media were significantly different after 21 days, using the values obtained for T_1 and T_2. Using the T_3 and T_4 values, the cultures were significantly different after 15 days. These results are discussed further in Section 7.4.

As different indices of tapering give different results, several points must be considered when selecting the most useful index for a particular purpose. The two indices T_1 and T_3, which do not involve percentages (p.73,80) may be used to compare tapering of one strain cultured under different conditions; while the two indices T_2 and T_4, which take percentage change in diameter into account, can be used for comparing the response of one or several strains to different conditions. The latter therefore are the most versatile.

Using T_1 and T_2 (which involve only two parameters) could produce the same value of tapering for trichomes of different lengths and a certain amount of information is lost. Using T_3 or T_4 overcomes this problem, but imposes others associated with a possible relationship

between trichome length and tapering. Such a relationship has important implications on the present work, as the organisms which were measured, even within one batch culture flask, showed a considerable range of trichome lengths (and presumably ages). If tapering is found to be dependent on trichome length, then great care would be needed in obtaining cultures of a more uniform age (Section 3.21).

A further problem which became apparent when making detailed measurements of individual cells in single filaments was that the concept of tapering based on a cone is an over-simplification (Section 3.22). In spite of these difficulties the indices discussed above provide a relatively quick method for obtaining a quantitative value of tapering and a useful way of comparing the influence of environmental factors on tapering. The effect of combined nitrogen on tapering is discussed in Section 7.4 and the influence of certain factors on hair development in Section 7.6.

7.3 The hair

The characteristics of the hair of members of the Rivulariaceae have been described in Section 1.32. Hairs have frequently been described in qualitative or semi-quantitative terms (Sections 3.3,5.42) yet for comparative work a measurement of hair length would be more useful. In order to quantify information related to the hair, it has been suggested (Section 3.3) that an actual measurement of the length of the colourless region of the hair be made.

7.4 The effect of combined nitrogen on tapered filaments

In - N medium, *C. brevissima* has been shown to taper from

6.0 to 4.5 μm, the trichomes being approximately 100 μm long. In the presence of NH_4-N, the trichomes became long and parallel, having a diameter of 4.5 μm. A similar response to the presence of NH_4-N has been described for Calothrix and Gloeotrichia (Fay, Stewart, Walsby and Fogg 1968). A different response however, was observed in Calothrix viguieri. This species had the characteristic tapered appearance of the Rivulariaceae in - N medium; while in the presence of NH_4-N, some trichomes became parallel and others were tapered towards both ends.

C. viguieri showed a similar response in medium containing NO_3-N (Section 4.32). Tapering was reduced from 59.2 to 21.8 using the tapering index T_2, and from 35.7 to 16.9, using the index T_4. C. scopulorum remained tapered when cultured in the presence of NO_3-N but the difference between the diameter of the basal and apical cells was reduced. After growth in medium + NO_3-N, the value of T_2 was reduced from 60.0 to 22.2, and the value of T_4 from 58.7 to 27.8.

The change in the value of T_2 for both C. viguieri and C. scopulorum indicates a decrease in the percentage difference in diameter between the basal and apical regions of trichomes of approximately 40%. The T_4 values for both strains were reduced to half their original values. It would be interesting to find out whether other strains showed similar numerical changes under these conditions. Apart from causing changes in the tapering of trichomes, the presence of combined nitrogen (NH_4-N or NO_3-N) resulted in a decrease in the percentage of trichomes with heterocysts. The percentage of trichomes with heterocysts was reduced to less than 5%

in C. brevissima (at an initial NH_4-N level of 24 mg l^{-1}) and to between 10-30% in C. viguieri, cultured under similar conditions. In the presence of NO_3-N, the percentage of trichomes with heterocysts in cultures of C.viguieri and C. scopulorum was reduced to 5-10% in 12-15 days.

In conjunction with these changes in morphology, in the presence of combined nitrogen, changes in the rate of nitrogen fixation in + N and - N media were studied. (Sections 4.2, 4.3). In all cultures grown in + N media, there was a considerable decrease in the rate of nitrogen fixation. The rate of nitrogen fixation in C. viguieri cultured for 21 days in medium in which the initial level of NH_4-N was 14 mg l^{-1} was 2 nmoles C_2H_4 h^{-1}mg $alga^{-1}$. This value was approximately a tenth of the rate in - N medium (21.5 nmoles C_2H_4 h^{-1} mg $alga^{-1}$).

In the presence of NO_3-N the rate of nitrogen fixation of both C. viguieri and C. scopulorum was very low; varying between 0.4-1.5 nmoles C_2H_4 h^{-1}mg $alga^{-1}$ and 1.28- 1.3-3.8 nmoles C_2H_4 h^{-1}mg alga, for the two strains respectively. In - N medium, the nitrogen fixation rates varied considerably during the growth of the strains. The rate of fixation in C. viguieri was relatively high for the first 18 days of growth, reaching a maximum of 24 nmoles C_2H_4 produced h^{-1} mg $alga^{-1}$, after 15 days. Following this there was a gradual decrease in the rate of fixation. In C. scopulorum the rate of nitrogen fixation was relatively high for the first five days of growth (21 nmoles C_2H_4 produced h^{-1} mg $alga^{-1}$), after which it decreased rapidly to a fairly constant, low level. Jones and Stewart (1969a)

suggested that this response in C. scopulorum could be attributed to the liberation of extracellular nitrogen into the growth medium.

It would seem therefore that the presence of combined nitrogen suppresses tapering in one of the three species of Calothrix studied and causes changes in the tapering of the other two species. In batch cultures of all three strains, the presence of combined nitrogen reduced the percentage of trichomes with heterocysts and reduced the rate of nitrogen fixation.

Changes in tapering and the absence of heterocysts from some trichomes have resulted in trichomes which possess morphological characters of genera other than the Rivulariaceae. C. vigiueri developed a Hammatoidea-like appearance in + N medium (Sections 4.2, 4.3) and trichomes of Calothrix scopulorum resembled Homoeothrix. Calothrix brevissima lost its taper and heterocysts, and if identified on the basis of its appearance in + N medium would not key-down to a member of the Rivulariaceae. The relevance of these observations is discussed further in Section 7.5.

7.5 Morphological variation and its implications on taxonomy

The problems involved in assigning specific names within a particular genus are evident from Section 7.4 and were encountered frequently during the ecological investigations. The results of experimental work on the effect of combined nitrogen on morphology (Sections 4.2, 4.3) illustrates the complexity of a taxonomy based primarily on morphology.

The material used for the experimental work (Chapter 4) was representative of the genus Calothrix, the trichomes having a basal heterocyst and tapering towards the apex. When three strains of Calothrix were cultured in the presence of combined nitrogen several morphological changes were observed. As described above and in Section 4.2, in + N media, a high proportion of the trichomes of C. brevissima had the appearance of parallel trichomes without heterocysts and morphologically were no longer representatives of the Rivulariaceae. Although trichomes of C. viguieri developed without heterocysts when cultured in + N medium, tapering was not lost. However trichomes of this strain became tapered towards both ends and resembled the genus Hammatoidea in appearance. Trichomes of Calothrix scopulorum developed without heterocysts but retained their taper and so resembled the genus Homoeothrix in appearance. Based on these results there appear to be several ways in which the presence of combined nitrogen in the growth medium can influence the development of tapering. Trichomes either loose their taper and become parallel or remain tapered. In the latter case the trichomes may become tapered towards one or both ends and/or the quantitative values assigned to the taper may decrease. Research carried out primarily by C. Sinclair during the later stages of this work, has suggested the generalisation that trichomes which possess a swollen base in - N medium tend to taper towards one or both ends in + N medium, and that those without a swollen base loose their taper.

There are several reports in the literature which indicate that variation in morphology can lead to confusion at the generic level of classification. Fogg, Stewart, Fay and Walsby (1973) stressed the difficulties involved in using the presence or absence of a heterocyst for taxonomic purposes. They described the inhibition of the development of heterocysts when Gloeotrichia filaments were grown on high levels of nitrogen. Under such circumstances, the filaments would probably be put into the non-heterocystous genera Homoeothrix or Leptochaete.

Separation of the genera Gloeotrichia and Rivularia on the basis of presence or absence of spores has been questioned by Singh and Tiwari (1970). They obtained a non-sporulating mutant strain of Gloeotrichia ghosei, in which they observed frequent heterocyst germination. The clone seemed to be a spontaneous mutant, and the loss of the spore appeared to be stable. On this basis, they questioned the validity of the genus Rivularia and suggested that species included under Rivularia were non-sporulating forms of Gloeotrichia.

It is evident therefore that the morphological characters used to distinguish not only species, but also genera show considerable variation and so lead to taxonomic confusion. As morphological characters are the only ones likely to be used at present, for taxonomic purposes, there is an urgent need for a closer study of the characters of Rivulariaceae and the factors which affect them.

7.6 The effect of certain factors on the development of the hair

Hairs were observed only once in a culture of Calothrix. On

this occasion, the observations were in agreement with those of Jones (1968) and indicated that hairs of C. scopulorum formed at low levels of salinity (Section 3.4). In comparison hairs were always present in at least some of the trichomes of every colony of Rivularia collected from the field. This material could not be cultured successfully and relatively little information about factors affecting the development of the hair has been obtained.

It was found that the % of the trichome which was colourless was greater when the level of PO_4 in the growth medium was low (Section 3.4). The hairs were also longer when Rivularia was kept in AD medium with a reduced level of iron as shown in Fig. 3.11, Section 3.4. Data of other workers, but included in a joint paper (Whitton, Kirkby, Peat and Sinclair 1973) have now shown that when cultured in AD medium, 3 out of 30 strains of Calothrix formed hairs (Section 3.4). Further experiments with C. viguieri, which did not produce hairs in AD medium, resulted in the formation of hairs under Fe-deficient conditions, in both + N and - N medium. Hairs were not produced in media deficient in Mo or Mn. The Fe-deficient cultures produced sheaths with a deep brown colouration. A similar feature was observed in the cultures of C. scopulorum which produced hairs (Section 3.4).

These few results suggest that hair formation may be promoted by low levels of certain nutrients. Reports on the hairs of certain green algae, although morphologically different to those of blue-green algae, suggest a similar function. Tupa (1974) has recently reported that hairs developed in Aphanochaete as the cultures aged and the medium became depleted, and also when the initial medium was nitrogen deficient. Promotion of hair development under

conditions of low nitrogen has been described in Stigeoclonium amoenum and Drapanaldia (Abbas and Godward 1973), and in Stigeoclonium farctum (Reynolds 1951).

Data from the literature (5.42) suggested that there was a greater tendency for strains of Calothrix and Rivularia, with spherical or hemispherical colonies, to produce hairs. Without exception in all such colonies described in the literature and observed by the author, the hair if present was near the outer surface of the colony. Colonies of this type have a relatively small surface area compared to volume and the development of hairs (with a large surface area) in such forms is likely to aid in absorption.

7.7 Problems associated with identifying Calothrix and Rivularia

As illustrated in Chapters 3 and 4, and stressed above (Sections 7.2, 7.3), two characters of the Rivulariaceae, the hair and the taper, show considerable morphological variation. During observations of trichomes of Rivularia, individual trichomes within a colony differed from each other in a number of ways. Using standard floras (Tilden 1910, Geitler 1932 and Desikachary 1959), it was rarely possible to arrive at a satisfactory binomial. An alternative identification scheme was developed in order to reduce the subjective element in identification and to increase the likelihood of different workers giving one colony the same binomial. The method devised has been described in Sections 5.2 and 5.3 and the key produced has used information from all of the characters

described in Section 5.2. Such an approach has been advocated by Womersley (1946) who described species of Rivularia as being far from satisfactory taxonomically. He stressed that considerable variation could be shown in different habitats and that in placing any particular specimen, attention should be focused on all the features used in separating the species rather than any single one.

The information used for identification purposes has been recorded in a standard way and used with the program 'IDENTIFY' (Section 5.4). Whitton (1969) suggested that considerable information has already been lost because workers have frequently used different media and growth conditions in laboratory experiments. It is hoped that by adopting standard recording procedures and laboratory techniques, that data will be collected in a way which stimulates discussion and comparison of results.

The results obtained using the 'simultaneous key' (Section 5.4) differ considerably from those obtained using the conventional floras. This is to be expected when using two such differing systems, and as pointed out in Section 5.4, one of the main reasons for these differences is likely to be the weighting of characters in the conventional floras. It was felt that a more intensive study of the individual characters used in taxonomy is required, before the subjective element of weighting is introduced into numerical keys. Once more information is available it is hoped that descriptions of characters will be extended to include biochemical, physiological and ultra-structural, as well as morphological ones.

The results of using 'IDENTIFY' to key down each known species in turn (Section 5.411) give support to the claim that several members of the Rivulariaceae are difficult to distinguish. Using 'IDENTIFY' to give a name to each of the tapered trichomes collected and coded (Section 5.412) it was found that within one colony, several 'named species' were represented. Although it seems possible that the 'species' of Rivularia and Calothrix may need to be revised to help overcome the present confusion, there is little reason to change specific names until more is known about the cause and extent of variation.

7.8 The ecological significance of Rivularia

One of the interesting facts to emerge from this work is that in certain streams or parts of streams the characteristic hemispherical colonies of Rivularia form a conspicuous and sometimes dominant part of the flora. In nearby or downstream sites, the colonies were frequently absent. Within the colony, individual trichomes were arranged radially and the majority of trichomes possessed long colourless hairs. Attempts to culture such colonies were largely unsuccessful and new colonies were observed on only one occasion. As described in Section 3.4, relatively long hairs were present in colonies from Gordale Beck which were cultured in AD medium with low phosphate (1.55 mg l^{-1}P and 31.0 mg l^{-1} P). The difficulties encountered in culturing these organisms may possibly be due to (1) the presence, in or on the colonies, of other blue-green algae which grew quickly under the laboratory conditions used,

(2) the wrong choice of media (3) the physical conditions imposed in batch culture. In this respect it is interesting to notice that the majority of the axenic and uni-algal cultures of Rivulariaceae present in the Culture Collections belong to the genus Calothrix. This genus does not grow as a conspicuous hemispherical colony and although the trichomes of many species have been reported to form hairs in the field (Section 5.42), hairs were observed once in culture.

Rivularia has a rather discontinuous distribution, being present in certain streams which drain limestone areas but absent from neighbouring streams. In particular, Rivularia was abundant in the upper reaches of streams and in many, but not all cases, where side flushes emerged from the ground. The results of the water analyses (Section 6.3) indicate that considering all of the sites at which Rivularia occurred there were relatively high levels of pH, Ca and Zn and relatively low levels of OD_{420}, Na, Cu, Fe and Al (Section 6.31). However these trends varied in different areas. The Zn result in particular should be treated with caution as this was only observed in one stream and may reflect a sampling bias. It seems probable that two features of Rivularia which have been studied here may bear considerable relevance to the ecology of the genus. Firstly, the colony form of Rivularia has a relatively small surface area : volume ratio. This may help withstand desiccation or may be a growth form suited to the flow conditions (Picken 1936).

The presence of a long colourless hair at the apex of the individual trichomes offers a large surface area to the surroundings. It has been suggested (Section 7.6) that this feature may compensate for the small surface area: volume ratio of the colony. Culture work (Section 3.4) has shown that hairs are relatively long in the presence of low levels of certain nutrients i.e. PO_4 and Fe ⁺⁺. At such times the large surface area : volume ratio of the hairs would be advantageous.

Secondly there is evidence that Rivularia from Teesdale, one of the areas included in this study, fixes atmospheric nitrogen (unpublished data M.K. Hughes). Information available to the author also suggests that the levels of combined nitrogen in the streams in this area in which Rivularia occurred were low (pers. comm. B.A. Whitton). Under such circumstances the ability to fix atmospheric nitrogen would be advantageous to the alga. Nitrogen fixation has been reported for a number of Rivulariaceae (Section 1.26). Jones and Stewart (1969a) stressed the importance of the contribution of marine Calothrix species to the nitrogen budget of rocky shores and it seems possible that Rivularia may have a similarly important role in upland streams.

7.9 Concluding remarks

It is evident from this work that if morphological characters are to form the basis of the identification of the blue-green algae, they must be studied in much greater detail. This is necessary in order to define the range of variation for each character and to show

how the characters vary in response to changes in external conditions.

The present study has illustrated that two characters of members of the Rivulariaceae, the taper and the hair, both show considerable morphological variation. The author has suggested ways of quantifying changes in these characters. The tapering indices developed were based on the simple shape of a cone and therefore assume that changes in diameter of a trichome occur at a uniform rate. This relatively simple concept has been used successfully to quantify the descriptions of the shapes of individual trichomes. The strains used during this work showed a considerable range in values of tapering indices. The range of values which would be obtained during the development of tapered trichomes from parallel hormogonia was not studied. If such studies are carried out in the future, it will be necessary to take into account the fact that the indices developed here give a simplified description of tapering. In many cases tapering does not occur gradually along the trichome, and detailed measurements of the cells in one trichome will be needed.

The tapering indices were also used with material in batch culture, to describe changes which occured in response to changes in environmental conditions. A value of tapering was obtained by measuring 30 trichomes at random and then finding the mean value. Ideally future workers should aim to use continuous cultures in which the trichomes are all of the same age. If batch cultures are used it will be necessary to measure selected trichomes which are all at the same stage of development.

A quantitative value for hair length was obtained by direct measurement and this was expressed as a percentage of total trichome length for comparative purposes. The problems encountered during this part of the work were associated with difficulties of observing hairs in culture. Future workers will need either to culture field material successfully or to discover the conditions under which the trichomes of cultured material produce hairs. If the first of these is attempted, it is suggested that material from either Red Sike of Gordale Beck may be relatively easy to culture. In view of more recent work, undertaken primarily by C. Sinclair, it seems likely that the second approach might prove the more informative particularly in terms of discovering which factors affect the development of a hair. It may then be possible to find out whether similar factors affect hair formation in the field situation.

The results of this work have emphasised the fact that the Rivulariaceae are difficult to identify at the generic as well as the specific level, using information from morphological characters. However, in practice, as these characters are the ones likely to be used by the majority of workers there is considerable scope for future research. It is to be hoped that workers studying morphological characters will adopt standard recording procedures and experimental procedure practices which will encourage discussion and comparison of data.

SUMMARY

A study has been carried out on the biology of tapering and the hair in two genera of the Rivulariaceae, Calothrix and Rivularia. A broad approach was adopted which involved four separate fields of study. These were an attempt to quantify the two characters, experimental studies to find specific causes for tapering and hair development, a computer study of characters in the literature and a field study to find any correlations between water chemistry and the occurrence of one of the genera (Rivularia).

As the definition of tapering and the hair was vague in the literature a prerequisite to experimental work was devising quantitative ways of describing these characters. Tapering, a gradual reduction in trichome diameter was fairly difficult to express in such a manner, however four indices of tapering have been suggested. These are:

$$T_1 = B-A$$

$$T_2 = \frac{B-A}{B} \times 100$$

$$T_3 = \frac{B-A}{L} \times 100$$

$$T_4 = \frac{\frac{B-A}{B} \times 100}{L}$$

where B = basal width, A = apical width and L = trichome length. Hair cells are colourless and frequently elongated and an actual measure of the length of the trichome which is composed of colourless cells has been used as a means of recording hair length quantitatively.

The experimental work has been concerned mainly with the effect of combined nitrogen on tapering. The results have shown that at relatively high initial levels of NH_4-N (29.2 mg l^{-1}) tapering did not develop in Calothrix brevissima or C. viguieri. In the case of C. viguieri, at a lower initial concentration of NH_4-N (14 mg l^{-1}) the trichomes were tapered towards both ends and developed a Hammatoidea-like appearance. In both species, at the highest initial concentrations of NH_4-N, heterocysts did not develop, and the rate of acetylene reduction (which was measured in C. viguieri) decreased considerably. In medium + NO_3-N (140 mg l^{-1}) the percentage of trichomes of C. viguieri which tapered decreased gradually during 30 days growth. At the higher levels of NO_3-N C. viguieri developed the Hammatoidea-like appearance which had also been observed in medium with NH_4-N. C. scopulorum showed a similar decrease in the percentage of trichomes which tapered, however this species did not become Hammatoidea-like and some of the trichomes were slightly tapered. In both strains the percentage of trichomes with heterocysts and the rate of acetylene reduction both decreased in the presence of combined nitrogen. More recent results obtained in conjunction with other works has now suggested that there may be a link between the appearance of the basal region of the trichome in - N medium and the appearance of the trichome in + N medium.

Hairs were observed once in laboratory culture, in C. scopulorum cultured in V_{37} medium at 5-20‰ salinity. Rivularia collected from the field frequently possessed hairs but was difficult to culture. The

results obtained suggested that the hairs of Rivularia were relatively long when it was cultured in a modified Allen and Arnon's medium with fairly low phosphate(initially 1.55-15.5 mg l^{-1}). The hairs were also relatively long when initial levels of Fe in the medium were reduced from 4 mg l^{-1} to 1 mg l^{-1}.

Information from the literature and summarised using a computer program, suggested that the possession of hairs appeared to be associated with the development of a spherical or hemispherical growth form. There was also a tendency for species of Calothrix and Rivularia which possessed a yellow-brown sheath to possess hairs.

The practical necessity of assigning names to material was particularly evident while collecting field material. Use of standard floras resulted in considerable ambiguity and therefore an alternative objective technique was devised. This employed the concept of a simultaneous key in which all of the characters of an organism are used in its identification.

A brief ecological survey was undertaken in order to find possible correlations between the presence of Rivularia and certain aspects of water chemistry. The areas studied were predominantly limestone regions and although not widespread, Rivularia was abundant and occasionally dominant in certain of these streams. A comparison of the water chemistry of streams with Rivularia and neighbouring streams without Rivularia showed that in general, Rivularia occurred at sites with relatively low OD_{420} and relatively high Ca and Mg. The levels of Zn, Cu, Mn, Fe, Al, and Pb tended to be lower in streams with Rivularia, but in some cases colonies were found in streams with fairly high levels of Zn and Fe. Colonies were found mainly in the

upper reaches of streams and often where springs emerged from the limestone. A microscopic examination of *Rivularia* from a number of sites has lead to the suggestion that, material from two streams in northern England (Red Sike, Upper Teesdale and Gordale Beck, Malham) may be suitable for future laboratory work.

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APPENDIX I

Authorities for Rivulariaceae included in this work

species	authority	date	reference
Calothrix adscendens	Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot., VII, 3, 365
C. aeruginea	(Kützing F.T.) Thuret G.	1875	Ann. Sci. nat. Bot., VI, 1, 10
C. aeruginosa	Woronichin N.N.	1923	Not. syst. Inst. Crypt. Horti. Bot. Petropol. 2, 115
C. africana	Schmidle W.	1901	Engler's Bot. Jb. 30,249
C. antarctica	Fritsch F.E.	1912	Natn. Ant. Exped. nat. Hist. 6, 36
C. atricha	Frémy P.	1930	Arch. Bot. 3, 261
C. bharadwaja	De Toni J.B.	1907	Syll. Alg. Myxophyceae
C. bossei	Frémy P.	1930	Arch. Bot. 3, 255
C. braunii	Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 3, 368
C. breviarticulata	West W. & West G.S.	1897	J. Bot. 35, 240
C. brevissima	West G.S.	1907	J. Linn. Soc.Bot. Lond. 38, 180
C. calida	Richter P.	1898	Gen. Plant. III, 2, 388
C. castellii	(Massalongo) Bornet E. & Flahault C.	1886	Ann. Sci.nat. Bot. VII, 3, 369
C. clavata	West G.S.	1914	In: Fuhrmann & Mayor, Voyage d' explor. Colombie, 1019
C. clavatoides	Ghose S.L.	1927	J. Burma Res. Soc. 17, 253
C. columiana	West G.S.	1914	Mem. Soc. Neuchatel. Sci. nat. 5, 1019
C. confervicola	(Roth) Agardh C.A.	1824	Syst. Alg. 70
C. conica	Gardner N.L.	1927	Mem. New York bot. Gdn 7, 66
C. consociata	(Kützing F.T.) Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 3,315

C. contarenii	(Zanardini G.) Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 3, 355
C. crustacea	(Schoub.) Thuret G.	1878	In: Bornet & Thuert, Notes Algol. 1,37
C. cylindrica	Frémy P.	1924	Revue algol. 1, 37
C. desertica	Schwabe G.H.	1960	Öst. Bot. Zeit. 107, 281-309
C. doliochomeres	Skuja H.		
C. donellii	(Wolle F.) De Toni J.B.	1907	Syll. Alg. Myxophyceae V, 629
C. elenkinii	Kossinskaja K.K.	1924	Not. syst. Inst. Crypt. Horti Bot. Petroplo. 3, 11
C. epiphytica	West W. & West G.S.	1897	J. Bot. 35, 290
C. evanescens	Gardner N.L.	1927	Mem. New York bot. Gdn 7, 69
C. fasciculata	Agardh C.A.	1824	Syst. Alg. 71
C. flahaultii	Frémy P.	1927	Arch. Bot. 5
C. floccosa	(Woronichin N.N.) Geitler L.	1925	Süßw.-Fl. , 12, 228
C. fullebornii	Schmidle W.	1902	Engler's Bot Jb. 32,62
C. fusca	(Kützing F.T.) Bornet E. & Flahault C.	1866	Ann. Sci. nat. Bot. VII, 3, 364
C. fusco-violacea	Crouan	1859	Ann. Sci. nat. Bot. VII, 4, 291
C. gardneri	De Toni J.B.	1907	Syll. Alg. Myxophyceae V
C. geitonos	Skuja H.		
C. gelatinosa	Fritsch F.E.	1923	Trans. R. Soc. S. Afr. 11, 375
C. ghosei	Bharadwaja		

C. gloeocola	Skuja H.		
C. goetzei	Schmidle W.	1901	Engler's, Bot Jb. 30, 248
C. gracilis	Fritsch F.E.	1912	Natn. Antarctic Exped. nat. Hist. 6, 37
C. intricata	Fritsch F.E.	1912	Natn. Antarctic Exped. nat. Hist. 6, 36
C. javanica	de Wilde		
C. juliana	(Meneghini G.) Bornet E. & Flahault C.	1886	Ann Sci. nat. Bot. VII, 3, 348
C. kawraiskyi	Schmile W.	1897	Alg. Hochseen Kaukasus 9
C. kossinskajae	Poljansky V.I.	1927	Arch. Russses Prot. 6, 70
C. kuntzei	Richter P.	1898	In: Küntze Rev. Gen. Plant. III,2,388
C. linearis	Gardner N.L.	1926	Rhodora 28, 3
C. marchica	Lemmermann E.	1914	Abh. Nat. Ver Bremen 23, 249-261
C. minima	Frémy P.	1924	Revue algol. 1,37
C. minuscula	Webber van Bosse A.	1913	Siboga. Exped. 59a, 42
C. parasitica	(Chauvin) Thuret G.	1875	Ann. Sci.nat. Bot. VI, 1, 381
C. parietina	Thuret G.	1875	Ann. Sci. nat. Bot. VI, 1, 381
C. parietina var. africana	Fritsch F.E.	1923	Trans. R. Soc. S. Afr. 11, 375
C. parva	Ercegovic A.	1925	Acta bot. Inst. Bot. Zagreb. 1, 94
C. pilosa	Harvey W.H.	1858	Nereis Bor. Am. 3, 106
C. prolifera	Flahault C.	1886	Ann. Sci. nat. Bot. VII, 3, 361
C. pulvinata	(Mertens) Agardh C.A.	1824	Syst. Alg., 71

C. ramenskii	Elenkin A.A.	1922	Mot. syst. Inst. Crypt. Horti. Bot. Petropol. 1, 9
C. robusta	Setchell W.A. & Gardner N.L.	1918	New Pacific Coast Alg. Univ. Calif. Publ. Bot. 40, 473
C. sandviencis	(Nordstedt C.F.) Schmidle W.	1897	Flora 84, 170
C. santapaui	Gonsalves A. & Kamat N.D.	1960	J. Bombay nat. Hist. Soc. 57, 454-456
C. scopulorum	(Weber & Mohr) Agardh C.A.	1824	Syst. Alg. 70
C. scytonemicola	Tilden J.E.	1910	Minnesota Algae 1, 265
C. simplex	Gardner N.L.	1927	Mem. New York bot. Gdn 7, 68
C. simulans	"	"	"
C. sphaeospora	Prasad B.N. & Srivastava P.N.	1965	Phykos 4, (2), 83-85
C. stagnalis	Gomont M.	1895	J. Bot. 9, 197-202
C. stellaris	Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 3, 365
C. submarchica	Archibald C.G.M.	1967	Nova Hedw. 13, 389-395
C. tenella	Gardner N.L.	1927	Mem. New York bot. Gdn 7
C. thermalis	(Schwabe) Hansgirg A.	1864	Ost. bot. Z. 34, 276-284
C. viguieri	Frémy P.	1929	Arch. Bot. 3, 252
C. violacea	(Wolle F.) De Toni J.B.	1907	Syll Alg. Myxophyceae V, 619
C. vivipara	Harvey W.H.	1858	Nereis Bor-Am. 3, 106
C. weberi	Schmidle W.	1899	Hedwigia 173
C. wembaerensis	Hieronymus G. & Schmidle W.	1898	in Kirchner's Engler-Prantl Nat Pfl. Fam. 1, 87
Rivularia aquatica	de Wide	1897	Alg. rapp. J. Mas. Ann. Buitenz. Suppl. 1, 40
R. atra	Roth A.W.	1806	Catalecta bot. 3, 340

R. australis	Harvey W.H.	1864	Some Acc. Mar. Bot. Austr. 566
R. beccariana	Bornet E. & Flahault C.	1886	Ann Sci. nat Bot. VII, 3, 356
R. biasolettiana	Meneghini G.	1841	In: Zanardini Synops. Alg. in mari Adriat. collect. R. Acad. Sci. Torino 4, 42
R. borealis	Richter P.	1897	Bibl. Bot. 7,4
R. bornetiana	Setchell W.A.	1895	Bull Torrey bot. Club 22, 426
R. bullata	Berkeley M.J.	1833	Glean Brit. Alg. 8
R. dura	Roth A.W.	1802	Neue Bretr, Bot. 273
R. globiceps	West G.S.	1907	J. Linn. Soc. Bot. Lond 38, 102
R. hansgirgi	Schmidle W.	1900	Hedwigia 173
R. haematites	Agardh C.A.	1824	Syst. Alg. 26
R. mamillata	Setchell W.A. & Gardner N.L.	1918	New Pacific Coast Alg. Univ. Calif. Publ. Bot. 475
R. manginii	Frémy P.	1931	'Travaux crypt. dedies a Louis Mengin' 103-8
R. mesenterica	Thuret G.	1875	Ann. Sci. nat. Bot. I, 6, 372-382
R. minutula	(Kützing F.T.) Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 4, 348
R. nitida	Agardh C.A.	1817	Disp. Alg. Suec. 41
R. peguana	Zeller G.H.		
R. planctonica	Elenkin A.A.	1921	Nachr. Petersb. bot. Gdn 16
R. polyotis	(Agardh) Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 4, 360
R. rufescens	Kaegeli C.	1849	In: Kütz., Spec. Alg. 342
R. viellardi	(Kützing) Bornet E. & Flahault C.	1886	Ann. Sci. nat. Bot. VII, 4, 343-373

APPENDIX II

Information on the morphology of *Calothrix* and *Rivularia* (from Tilden 1910, Geitler 1932 and Desikachary 1959).

Number	Organism	Physiognomic form	Colony height or filament length	Calcification
1.	C. adscendens	Filaments scattered or gregarious	1 mm (filament)	-
2.	C. aeruginea	Layer, on other algae	0.5 mm (filament)	-
3.	C. aeruginosa	Few filaments intertwined (2-3)	150 µm (filament)	-
4.	C. africana	Few filaments together	-	-
5.	C. antarctica	Filaments solitary or few together	≤ 300 µm (filament)	-
6.	C. atricha	Filaments solitary or in groups of 2-6	100 µm (filament)	-
7.	C. bharadwaja	Filaments usually in groups of 3-7	420 µm (filament)	-
8.	C. bossei	Filaments clustered and forming thallus	1 mm (filament)	-
9.	C. braunii	Filaments caespitose	0.5 mm (filament)	-
10.	C. breviarticulata	Filaments solitary or gregarious	380 µm (filament)	-
11.	C. brevissima	Filaments together	53-94 µm (filament)	-
12.	C. calida	Thallus flattened and expanded	6 mm thick (Thallus)	Sometimes
13.	C. castellii	Cushion-shaped and widely expanded	4 - 8 mm	-
14.	C. clavata	Filaments solitary or few together	100 µm (filament)	-
15.	C. clavatoides	Trichomes solitary or in small groups	≤ 200 µm (filament)	-
16.	C. columbiana	Filaments clustered	350 µm (filament)	-
17.	C. conica	Not recorded	125 µm, mostly 45-65 µm	-
18.	C. confervicola	Filaments gregarious, stellately fasciculate	2-3 mm (filaments)	-

Number	Texture	Arrangement of trichomes	Tapering	Hair
1.	-	-	Present, gradual	Present
2.	-	-	Abrupt at base of hair	Present
3.	-	Filaments intertwined at bases	End cell tapered	Absent
4.	-	Filaments contorted	Present, gradual	Present
5.	-	Filaments solitary or loosely appressed	Present	Absent
6.	-	Filaments erect and curved to some extent	Gradual taper, end cell pointed	Absent, but may be chance
7.	-	Filaments straight or slightly bent	Present	Long
8.	-	Filaments interwoven	Present, gradual	Absent
9.	velvety	Filaments densely crowded, parallel or slightly curved	Present	Long
10.	-	-	Present, gradual	-
11.	-	Many occur together	None or very little	Absent
12.	Dry and crustaceous	Filaments aggreagated and interwoven, flexuous	Present	Long
13.	Spongy	Filaments densely crowded, flexuous and curved	Present, gradual	Very long
14.	-	Filaments straight or slightly bent	Present	Present
15.	-	Filaments straight or curved	Present	Present
16.	-	Filaments erect or curved	Present	-
17.	-	Filaments straight or curved	Present, near apex	Absent
18.	-	Filaments in small tufts	Present, abrupt near hair	Present

Number	Shape of basal cell	Cell width	Base of trichome swollen or not	Cell indentations
1.	Longer than broad	12 µm (in centre)	No	Slightly
2.	Shorter than broad	7-9 µm	Hardly	Yes (some)
3.	Shorter than broad	8.5 µm	-	Yes
4.	Almost as long as broad	5 µm	No	At base
5.	Always broader than long	6-8 µm	-	Slightly in upper parts
6.	½-2 times as long as broad	6-7 µm	-	Yes
7.	As long as broad	6.3 µm	-	Yes
8.	Squarish or ½ as long as broad	>10 µm	Yes	Slightly in upper regions
9.	Shorter than broad	6-7 µm	Yes	Yes
10.	4-5 times shorter than broad (disc-shaped)	8.5 µm	-	-
11.	Nearly as long as broad	3.8-5 µm	No	Hardly
12.	As long as broad	3-6 µm	-	-
13.	2-4 times shorter than broad	12-13 µm (filament)	Yes	Yes
14.	Discoid	5-5.5 µm	Yes	Slightly
15.	Discoid	10-13 µm	Yes	Yes
16.	As long as broad or almost as long	6-8 µm	No	No
17.	Cylindrical, as long as broad	4.8-5.6 µm	Filaments swollen at base	In upper parts
18.	4-5 times shorter than broad	10-18 µm	Sometimes	Sometimes

Number	Width of base compared to heterocyst	Number of heterocysts	Position of heterocysts	Shape of basal heterocyst
1.	Equal	Single	Basal	Spherical
2.	Equal	1-2 basal, few or none intercalary	Basal and intercalary	Rounded
3.	Heterocyst slightly wider (8-9 μm)	Presumed single	Presumed basal	Spherical
4.	Heterocyst wider (8.5 μm)	Single	Basal	Hemi-spherical
5.	Heterocyst wider (10 μm)	Single, seldom	-	Elliptical or flattened
6.	-	1-2	Basal	Spherical
7.	Equal or heterocyst narrower (4.2-6.3 μm)	Basal single Intercalary, single or in pairs	Basal and intercalary	Spherical or sub-spherical
8.	-	Single	Basal	Flattened or hemispherical
9.	Equal or heterocyst narrower	Presumed single	Basal	Subspherical
10.	Heterocyst wider	Single	Basal	Hemispherical
11.	Equal or heterocyst wider	Single, seldom two	Basal	Nearly spherical
12.	-	Several	Basal and intercalary	Spherical or Quadrate
13.	-	Single	Basal	Subspherical
14.	Equal	Single	Basal	Hemispherical
15.	Trichome slightly wider	Single	Basal	Globose
16.	Equal or heterocyst narrower	Single	Basal	Spherical
17.	Equal	Single	Basal	Spherical or sub-spherical
18.	Equal	1-2	Basal	Rounded

Number	Shape of sheath	Texture of sheath	Width of sheath	Colour of sheath	Sheath lamellations
1.	Wide in middle, tapers beyond hair	Gelatinous	3-6 μm	Colourless	Present
2.	Parallel	Gelatinous	1-1.5 μm	Colourless, yellow in lower parts	Absent
3.	-	-	< 1.5 μm	Colourless	Absent
4.	Frayed at top	-	6 μm	Red-brown at base, becoming colourless	Present
5.	Diffluent at apex	-	2-4 μm	Yellow	Present
6.	Open at apex	-	Thin	Colourless	Absent
7.	Close to trichome, open at apex	-	Thin	Colourless	-
8.	Close to trichome	-	Thin	Colourless	Scarcely lamellated
9.	Close to trichome	-	Narrow, 1.5 μm	Colourless	Absent
10.	Tapered above tip of trichome	-	Thick 8 μm	Brownish-black in older parts	Present
11.	Close to trichome	Firm	Thin < 2 μm	Colourless	Absent
12.	Close to trichome, sometimes dilated	-	Thick 2-2.5 μm	Yellow-brown, colourless when young	-
13.	Close to trichome	Firm	Thin	-	Absent
14.	Close to trichome	-	Very thin ≤1.5 μm	Colourless	Absent
15.	Close to trichome	-	Thin ≤2.0 μm	Colourless	Absent
16.	Close to trichome	Gelatinous	Thin	Colourless	Absent
17.	Close to trichome	-	Thin 1 μm	Colourless	Absent
18.	Parallel and close to trichome	Gelatinous in upper parts	3.5 μm	Colourless or yellow-brown in lower parts	Sometimes

Number	Sheath lacerations	Spores	Habitat
1.	-	Absent	Freshwater
2.	-	-	Marine, on algae, iron pilings and wood
3.	-	-	In marine marsh, on larger algae
4.	-	Absent	Hot springs
5.	-	-	In standing water in Antarctic, in thallus of Phormidium fragile
6.	-	-	In standing water
7.	Absent	Spores single	In stagnant pond, on dead leaves
8.	-	Absent	In spring, on quartz rock
9.	Absent	-	On stones and plants in streams
10.	Present	-	Freshwater, on Vaucheria
11.	Absent	-	Epiphytic on plants in African lakes
12.	-	-	In warm water from geysir
13.	-	-	On shelves in greenhouse
14.	-	-	-
15.	-	-	In pond, Burma
16.	-	Spore single	-
17.	-	-	On tree trunks near Coame Springs
18.	-	-	Freshwater or in saltmarsh, or other alga

Number	Organism	Physiognomic form	Colony height or filament length	Calcification
19.	C. consociata	Filaments gregarious stellately fasciculate	0.5 mm (filaments)	-
20.	C. contarenii	Compact layer, orbicular	Filaments 1 mm long	-
21.	C. crustacea	Caespitose, widely expanded	1-2 mm long (filament)	-
22.	C. cylindrica	Layer	-	-
23.	C. desertica	Hemispherical thallus	0.5-4 mm	Absent
24.	C. dolichomeres	Caespitose colonies	1 mm long (filament)	-
25.	C. donnellii	Caespitose	-	-
26.	C. elenkinii	Filaments united in tufts	80-250 µm long	-
27.	C. epiphytica	Solitary or gregarious	250-350 µm long (filaments)	-
28.	C. evanescens	-	200-300 µm long (filaments)	-
29.	C. fasciculata	Caespitose, expanded	2-3 mm long (filaments)	-
30.	C. flahaultii	Filaments in groups of 3-9	-	-
31.	C. floccosa	Thallus	400 µm long	-
32.	C. fullebornii	Filaments single or in groups, 10-12	-	-
33.	C. fusca	Filaments scattered or gregarious	2-3 mm (filaments)	-
34.	C. fuso-violacea	Gregarious and forming thallus	0.5 mm long (filaments)	-
35.	C. gardneri	Filament single	350-500 (-1000) µm	-
36.	C. geitonos	Caespitose colony	1 mm long (filaments)	-
37.	C. gelatinosa	Thallus	-	-

Number	Texture	Arrangement of trichomes	Tapering	Hair
19.	-	-	Presumed present	-
20.	Crustaceous	Densely crowded, parallel, erect, flexuous	Present	Long and slender
21.	Velvety	Densely, crowded, erect	Present	Long
22.	Slimey	Scattered, prostrate	Present	Curved hair
23.	Soft	-	Apical cell conical	Absent
24.	-	Erect, flexuous	Gradual	Long
25.	Gelatinous	Densely intertwined	Gradual	Long
26.	-	Intertwined	Present	Absent (not formed)
27.	-	Solitary or gregarious	Gradual	Thin
28.	-	Erect or bent	Present	Present
29.	Velvety	Erect, somewhat flexuous	Present	Present
30.	-	Erect and straight	Present	Short
31.	-	Filaments ramifying	Present	Present
32.	-	-	Gradual	-
33.	Gelatinous	-	Present	Long
34.	Velvety	-	Present	Short
35.	-	Erect	Tapered at apex	Short
36.	-	Rarely solitary, erect or flexuous in caespitose colony	Present	Long
37.	Gelatinous	Intertwined	Present	Absent

Number	Shape of basal cell	Cell width	Base of trichome swollen or not	Cell indentations
19.	Shorter than broad	12 µm	Filament swollen at base	Some
20.	Equal to or shorter than width	6-8 µm	Filament swollen at base	Sometimes in lower cells
21.	Shorter than broad	8-15 µm	Filament slightly thickened	Sometimes
22.	Shorter than broad	10-15 µm	Sometimes	Yes
23.	$\frac{1}{5}-\frac{1}{4}$ as long as broad	6-14 µm	Yes	Some
24.	1-2½ times as long as broad	7-8 µm	-	-
25.	4-5 times shorter than broad	Filament 6-12-(20) µm	-	-
26.	Quadrate or sometimes shorter than long	5-7 µm	Filament swollen at base	No
27.	Shorter than broad	3.5-4 µm	-	-
28.	$\frac{1}{3}$ as long as broad	Filament 7-8 µm	-	-
29.	2-3 times shorter than broad	8-12 µm	Slightly when young	-
30.	1½ times as long as broad	5.5-6.5 µm	-	No
31.	Longer than broad	4.7-9 µm	-	Yes
32.	Quadrate or $\frac{1}{2}-\frac{1}{4}$ as long as broad	6-10(14) µm	No	Yes
33.	Shorter than broad *	7-8 µm	Filament "bulbous"	No
34.	Shorter than broad	7-8 µm	Filament swollen	Yes
35.	Quadrate or ½ as long as broad	5-7 µm	Filament swollen	At base
36.	1-1½ times as long as broad	7-8 µm	No	Yes
37.	As long as broad	16 µm	-	No

* Longer than broad (Swellengrebel 1910)

Number	Width of base compared to heterocyst	Number of heterocysts	Position of heterocysts	Shape of heterocysts
19.	Equal	Single	Basal	-
20.	Trichome wider than heterocyst	1-2	Basal	-
21.	Equal or heterocyst slightly wider	Several	1-3,basal, many intercalary	Basal,spherical
22.	Heterocyst slightly wider	Single	Basal	Hemispherical kidney-shaped
23.	-	Single or several	Intercalary (rare)	Hemispherical
24.	Heterocyst wider (8-9.5 μm)	1-3	Basal	Round, hemi-spherical, barrel-shaped
25.	-	Basal,rarely intercalary	Basal and intercalary	-
26.	Equal,or heterocyst slightly narrower	Single	Basal	Sub-spherical
27.	-	Single	Basal	-
28.	Equal	Single	Basal	Hemispherical or spherical
29.	-	Several	Basal	-
30.	Equal	Single	Basal	Rounded cylinder
31.	Equal or heterocyst slightly narrower	Single or often in twos	Basal	Squarish or longer than broad
32.	-	Several	Basal and intercalary in series	-
33.	Equal or heterocyst wider	1-2	Basal	Widest in centre, flattened
34.	-	Single	Basal	-
35.	-	Single	Basal	Hemispherical
36.	Heterocyst wider (8-9.5 μm)	1-3	Basal	Rounded, hemi-spherical, barrel-shaped
37.	Heterocyst wider	Single	Basal	Broader than long

Number	Shape of sheath	Texture of sheath	Width of sheath	Colour of sheath
19.	Closed, funnel-shaped at apex	membranous	4.5-8.5 µm	Brownish, outer layers colourless
20.	Funnel-shaped	-	Thick (3.5 µm)	Colourless or yellow
21.	Dilated and expanded at apex	-	Thick (2-12 µm)	Colourless or yellow-brown
22.	Close	Gelatinous	2-3 µm	Colourless or yellow
23.	Close	-	Thick or thin	Golden at base
24.	Wider at base	Gelatinous	3 µm	Colourless
25.	Tapered, or open	-	Very thin	Colourless
26.	Close, open at apex	-	Thin	Colourless
27.	-	-	Thick	Colourless
28.	Close	-	Thin	-
29.	Dilated	Firm	Thick (2-6 µm)	Colourless, becoming yellow-brown
30.	-	-	Thin	-
31.	Diverging	Gelatinous	Thick (9-12 µm)	Colourless, base yellow-brown
32.	-	-	Thin (1.5-2.0 µm)	Colourless
33.	Diffluent at apex	Gelatinous	Thick (1.5-2 µm)	Colourless
34.	Close	-	Thin (1-2 µm)	Colourless
35.	-	Gelatinous	2-2.5 µm	Colourless
36.	Diffluental apex	Mucilagenous	3 µm	Colourless
37.	-	Gelatinous	5 µm	Colourless, later coloured

Number	Sheath lamellations	Sheath lacerations	Spores	Habitat
19.	-	-	-	On grass in salt marsh
20.	Present	-	-	On stones and wood, in sand
21.	In older filaments	-	In series	Marine, upper limit of water
22.	Two layers	-	Absent	On moist rocks
23.	Absent	-	-	Marine and freshwater
24.	Partly lamellated	-	-	-
25.	-	-	-	On wood in salt water
26.	Absent	-	-	Submerged rocks
27.	-	-	-	Freshwater, on *Tolypothrix*
28.	Absent	-	-	Standing water
29.	Present	-	-	On rocks, between tide marks
30.	Absent	-	-	In *Sphagnum* humps
31.	Present	-	-	On *Potentilla palustris* in stagnant lake
32.	Absent	-	-	In standing or flowing water
33.	Indistinct	-	-	Freshwater
34.	Absent	-	-	Marine, orbicular patches on *Punctaria*
35.	Absent	-	-	On building plinth
36.	Partly lamellated	-	-	On grass, in lake
37.	Indistinctly lamellated	-	Absent	Between moss, in tropical waters

Number	Organism	Physiognomic form	Colony height or filament length	Calcification
38.	C. ghosei	Filaments in groups	250 μm long	-
39.	C. gloeocola	Filaments solitary	650 μm long	-
40.	C. goetzei	Filaments forming crust	-	Present
41.	C. gracilis	Filaments in small bundles	400 μm long	-
42.	C. intricata	Filaments forming thallus	300 μm long (filaments)	-
43.	C. javanica	Filaments single in algal mucilage	-	-
44.	C. juliana	Filaments scattered or in thallus	2 mm long (filament)	-
45.	C. kawraiskyi	Forming mat		-
46.	C. kossinskajae	Sometimes hemi-spherical colony	200-340 μm long	-
47.	C. kuntzei	Crust	5 mm thick	Present
48.	C. linearis	-	350-500 (1000 μm)	-
49.	C. marchica	Solitary	-	-
50.	C. membranacea	Skin-like thallus	300 μm long	-
51.	C. minima	Many filaments together	100 μm	-
52.	C. minuscula	Filaments in groups	-	Present
53.	C. parasitica	Gregarious	0.5 mm long	-
54.	C. parietina	Filaments scattered or forming a crust	1 mm long	Sometimes

Number	Texture	Arrangement of trichomes	Tapering	Hair
38.	-	Filaments in groups, straight or bent	Apical cell pointed or rounded	-
39.	-	Filaments solitary	Present, gradual	Long
40.	-	Filaments densely crowded	Sudden taper	Long
41.	-	Filaments in small bundles	Apical cell pointed	No
42.	-	Filaments densely entangled	Present, gradual	Absent
43.	-	Single	Gradual	Thin
44.	-	Scattered or densely crowded	Present	Long and fragile
45.	-	Filaments densely crowded	Present	Long
46.	-	Filaments bent or parallel	Present (may be abrupt)	Long and slender
47.	Crustaceous	Irregularly fasciculate parallel or flexuous	Present	-
48.	-	Erect, mostly straight	Present	Short
49.	-	Filaments solitary	Gradual	Absent
50.	Papery	Curved, irregularly floccose	Gradual	Present
51.	-	Many filaments together	Present	-
52.	Calcified	Filaments in groups	Present	-
53.	-	Gregarious	Present	Long and flexuous
54.	-	Scattered or aggregated	Present	Thin Long

Number	Shape of Basal cell	Cell width	Base of trichome swollen or not	Cell Indentations
38.	Quadrate, slightly longer or shorter than broad	6.3-8.4 µm	-	Slightly constricted at cross walls
39.	-	5-6.5 µm	Filament base dilated	-
40.	½-3 times as long as broad	8 µm (5-9 µm)	-	-
41.	Shorter than broad or squarish	5 µm	Base filament swollen	Indented at base
42.	Quadrate or shorter than broad	5-6 µm	No	Slight
43.	Slightly broader than long	4-6 µm	No	Sometimes
44.	3 times shorter than broad	9-12.5 µm	Filament often thickened at base	-
45.	Rectangular, quadrate or ½ as long as broad	> 10 µm	Filament thickened at base	-
46.	Quadrate or longer than broad	3-6 µm	Filament swollen	Slight or not
47.	Hemispherical, disc-shaped, spherical	(10-11 µm)	Yes	-
48.	Squarish or ½ as long as broad	5-7 µm	Yes	At base
49.	Squarish or ½-¼ as long as broad	4-5.5 µm	-	Slight
50.	½ as long as broad	5-6 µm	Filament sometimes swollen	-
51.	½ as long as broad	8 µm	-	No
52.	Squarish or shorter than broad	3.6-4.5 µm	Filament distinctly swollen	-
53.	Cells shorter than broad	7-8 µm	Filament bulbous at base	Slight
54.	2-3 times shorter than broad	5-10 µm	Filament somewhat thickened	Sometimes

Number	Width of base compared to heterocyst	Number of heterocysts	Position of heterocysts	Shape of basal heterocyst
38.	Trichome broader 4.2-6.3 µm	Single	Basal	Longer than broad
39.	Heterocyst broader 6-7 µm	Single	Basal	Hemispherical or globose, sub-ovoid, truncate
40.	-	-	Intercalary	(2-3 times as long as broad)
41.	Heterocyst equal or slightly wider	Several	Basal, some intercalary	Subspherical
42.	Equal	Single, or several	Basal	-
43.	Trichome equal or just slightly broader	Not stated	Basal	Spherical
44.	Heterocysts absent	Absent	Absent	Absent
45.	-	Single	Basal	Hemispherical or longer than broad
46.	Equal	Single	Basal	Broader than long
47.	Heterocyst wider	Several	Basal and intercalary	Spherical or elliptical
48.	Basal cell wider	Single	Basal	Hemi-spherical
49.	Equal	Single	Basal	Spherical or hemispherical
50.	Equal	Single	Basal	As long as wide
51.	Basal cell wider	Single	Basal and intercalary	Hemispherical
52.	-	-	-	-
53.	Equal or basal cell slightly wider	Single or 2-3	Basal	Spherical or hemispherical
54.	Heterocyst slightly wider	Several	Basal, rarely intercalary	Wider at base or spherical

Number	Shape of sheath	Texture of sheath	Width of sheath	Colour of sheath
38.	-	-	Thin < 2.5 μm	Colourless
39.	Sometimes diffluent	-	1 μm	Colourless
40.	-	-	Very thin	-
41.	Close to trichome	-	Very thin 2 μm	Colourless
42.	Diffuse	-	Thin	Colourless
43.	Tapered	-	Wide	Colourless
44.	Close	-	Thin 1.5 μm	Colourless
45.	Close	-	Thin	Colourless
46.	Close and often closed	-	Thin	Colourless
47.	Close	-	Thick	Colourless or yellow
48.	-	Gelatinous	2-2.5 μm	Colourless
49.	Surrounding trichome	-	Thin	Colourless
50.	Close	-	Thin	Colourless
51.	Close	-	Thin	Colourless
52.	-	-	1.5-2 μm	Colourless
53.	Funnel-shaped	-	Thin 2-3 μm	Colourless
54.	Close ocreate	-	Thick	Yellow-brown

Number	Sheath lamellations	Sheath lacerations	Spores	Habitat
38.	-	-	-	Stagnant pool on *Chara*
39.	No	-	-	Lake, in thallus of *Nostocopsis*
40.	-	-	-	In hot springs
41.	-	-	1-2	-
42.	-	-	-	In standing water, in thallus of *Phormidium*
43.	No	-	Single or 2 together	In hot springs
44.	No	-	-	On wood and stones in shallow water
45.	-	-	-	Stagnant water
46.	Slightly lamellated	-	-	On *Cladophora*
47.	Present	-	-	In running water from hot spring
48.	No	-	-	In water pump
49.	-	-	-	In sheath of *Nostoc linearis*
50.	No	-	-	
51.	-	-	-	On *Dichothrix* sp.
52.	-	-	-	In standing water
53.	New layer forms at base of hair	-	-	On Nemalion
54.	Present	ocreate	-	On damp rocks

Number	Organism	Physiognomic form	Colony height or filament length	Calcification
55.	C. parietina var. africana	Spreading thallus	2 mm high	-
56.	C. parva	Filaments solitary or mixed with other algae	60-80 µm long	-
57.	C. pilosa	Caespitose	-	-
58.	C. prolifera	Expanded thallus	2 mm long (Filament)	-
59.	C. pulvinata	Sponge like thallus, tufts	2-3 mm long	-
60.	C. ramenskii	Single or groups of filaments	-	-
61.	C. robusta	Filaments in tufts	1-2 mm long	-
62.	C. sandviencis	Single or in groups	-	-
63.	C. santapaui	Single Filaments	-	-
64.	C. scopulorum	Filaments caespitose	1 mm long	-
65.	C. scytonemicola	Single filaments or small groups	-	-
66.	C. simplex	Layer	200-250 µm long	-
67.	C. simulans	Few together	200-250 (400) µm long	-
68.	C. sphaerospora	Sub-circular patches on agar	-	-
69.	C. stagnalis	Filaments gregarious	1 mm long	-
70.	C. stellaris	Filaments single or in groups	-	-
71.	C. submarchica	Filaments single or in groups	250-350 µm long	-
72.	C. tenella	-	60-90 µm long	-

Number	Texture	Arrangement of Trichomes	Tapering	Hair
55.	Firm	Filaments intertwined	Present	Absent
56.	-	-	Present	Short
57.	-	Intertwined at base, erect at apex	Apical cell hemispherical	No
58.	Velvety	Flexuous and curved	Present	Present
59.	Porous, sponge-like	Erect and flexuous aggregated, fasciculate	Present	Short
60.	-	Erect or slightly bent	Present	Present
61.	-	Firmly appressed at base	Abrupt taper	-
62.	-	Curved, single or in groups	Present	Long
63.	-	Straight or curved	Present	Long
64.	Velvety	Twisted	Present	Present
65.	-	Erect	Present	Present
66.	-	Straight and curved at base	Gradual	Present, long
67.	-	-	Present	Short
68.	-	-	Present	Present, long
69.	Papery	Sickle-shaped, gregarious, radial	Gradual	Present
70.	-	Straight or bent	Present	Present, thin
71.	-	Curved	Gradual	Absent
72.	-	Twisted	Present	Present

Number	Shape of Basal cell	Cell width	Base of trichome swollen or not	Cell indentations
55.	Shorter than broad or squarish	6.5-9 μm	Slightly	Mostly
56.	-	7-8 μm	Slightly	No, or slightly
57.	Quadrate or 2-4 times as long as broad	10-20 μm	-	-
58.	3-4 times shorter than broad	8-12 μm	Filament thickened at base	In parts
59.	2-3 times shorter than broad or squarish	8-12 μm	No	Hardly
60.	Shorter than broad	17-24 μm	Yes	-
61.	-	16-20 μm	-	No
62.	3-4 times longer than broad	3.5-5.5 μm	Filament thickened	Some
63.	Squarish or shorter than broad	5.1-5.4 μm	Thick layer of mucilage at base	No
64.	Squarish or shorter than broad	8-15 μm	Filament thickened	In narrow regions
65.	Longer than broad	7-8 μm	-	Yes,in regions
66.	Shorter than broad	10-14 μm	Yes, in older filaments	No
67.	Squarish or 3 times shorter than broad	6-7 μm	-	Basal cells slightly
68.	Spherical or sub-spherical	8-9.5 μm	-	-
69.	Subquadrate or longer than broad	6-9 μm	Filament thickened at base	Yes in regions
70.	$\frac{1}{2}$ as long as broad	6-7 μm	Filament swollen	Slightly
71.	Broader than long	8-10 μm	-	Yes
72.	Squarish or $1\frac{1}{2}$ times as long as broad or shorter than broad	4.8-6.2 μm	Filament slightly swollen	-

Number	Width of Base Compared to Heterocyst	Number of Heterocysts	Position of heterocyst	Shape of heterocysts
55.	Basal cell wider in mature filaments	Single or several	Basal, seldom intercalary	Spherical
56.	Heterocyst narrower or broader, 6-9 μm	Single	Basal	Spherical or hemispherical
57.	-	Several	Basal and intercalary	-
58.	-	Several or many	1-2 basal, intercalary scattered	-
59.	Equal	Single	Basal	Subspherical
60.	Broader than trichome	Single or several	Basal, sometimes intercalary	Bullet-shaped
61.	-	1-4	Basal	-
62.	Heterocyst wider	Single	Basal	Subspherical
63.	Heterocyst wider 5.8-6.4 μm	Single	Basal	Spherical or ellipsoidal
64.	Equal	1-3	Basal	Spherical, subspherical
65.	Heterocyst 8 μm in diameter	Usually 2	Basal	Globose
66.	Equal	Single	Basal	Hemispherical or elongated
67.	Equal 6.8-7.2 μm	Single	Basal	Spherical or hemispherical
68.	Heterocyst narrower 5.5-6.5 μm	Single or several	Basal, sometimes intercalary	Longer than broad
69.	Heterocyst wider	In pairs	Basal	Spherical or quadrate
70.	Equal	Single or 2-3 together	Basal	Hemispherical
71.	Heterocyst narrower 7.5-8 μm	Single	Basal	Broader than long
72.	Heterocyst narrower	Single	Basal	Spherical or hemispherical

Number	Shape of Sheath	Texture of Sheath	Width of Sheath	Colour of Sheath
55.	Parallel or diverging	-	Thick 5 μm	Yellow-brown and colourless
56.	-	Firm	< 2.5 μm	Yellow-brown
57.	-	Firm	Thick 5 μm	Orange or yellow-brown
58.	Dilated	Firm	Thick 3.5 μm	Upper regions colourless, lower ones yellow
59.	Parallel	Firm	Thick	Colourless or brown
60.	Sometimes funnel shaped	-	Thick Approx.8 μm	Colourless and brown at base
61.	-	-	Thick 7-8 μm	Colourless or yellow
62.	-	-	Thick	Colourless
63.	-	Firm	-	Colourless or yellow-brown
64.	Dilated in upper parts	-	Thick	Colourless or yellow-brown
65.	Not distinct	-	-	-
66.	Close and tapered	Firm, smooth	2-4 μm	Colourless
67.	-	Firm	Very thin	Colourless
68.	Envelopes whole trichome	Smooth	-	Colourless
69.	Close	Papery	Thin	Colourless
70.	Parallel with trichome	-	3 μm	Colourless
71.	-	Firm	Thin	Colourless
72.	Tapered	-	2.5-3 μm	Colourless

Number	Sheath lamellations	Sheath lacerations	Spores	Habitat
55.	Slight	Slight	-	On rocks
56.	Slightly	-	-	Damp rocks
57.	Absent	-	-	On rocks, marine pools
58.	Present	Ocreate frayed	-	On boards, wet with salt water
59.	Present	-	-	On wood, marine
60.	Present	-	-	In lake
61.	Present	-	-	Marine
62.	Present	-	Single, or pairs	Freshwater
63.	Distinct	-	1-3 in rows	In mucilage of other algae
64.	Present	-	-	On rocks, near high water mark
65.	-	-	-	Freshwater
66.	Absent	-	-	On stones, in stream
67.	Absent	-	1-3	In standing water on Oedogonium
68.	Absent	-	Singly or in series	In culture of soil
69.	-	-	Present	Marine and freshwater
70.	Present	Present	-	Stagnant tub water on Chara
71.	-	-	Absent	River Kowie, South Africa
72.	-	-	-	On rocks, amongst liverworts

Number	Organism	Physiognomic form	Colony height or filament length	Calcification
73.	C. thermalis	Expanded thallus	3 mm long	-
74.	C. viguieri	Flake-like thallus	150-200 (-300) μm long	Present
75.	C. violacea	Clusters of 10-12	-	-
76.	C. vivipara	Expanded thallus	3-5 μm long	-
77.	C. weberi	Filaments free-floating or attached	-	-
78.	C. wembaerensis	Caespitose	1 mm long	-

Number	Texture	Arrangement of trichomes	Tapering	Hair
73.	Mucus-like	Interwoven and densely crowded	Present	Long
74.	-	Interwoven, straight or bent	Gradual	Absent
75.	-	In clusters of 10-12	Present	-
76.	Velvety	Interwoven, erect or flexuous	Gradual	Present
77.	-	Irregularly coiled	Present	Present
78.	Gelatinous	In caespitose colony	Present	-

Number	Shape of Basal cell	Cell width	Base of trichome swollen or not	Cell indentations
73.	(Squarish) or 3 times shorter than broad	5-8 μm	-	In some regions
74.	Shorter than broad	10-(15) μm	Yes	Rarely
75.	2-4 times shorter than broad	7-9 μm	-	-
76.	Squarish or shorter than broad	9-15 μm	-	-
77.	A little longer than broad	5.1 μm	-	Slight
78.	½ - 1½ times as long as broad	8 μm	-	-

Number	Width of Base compared to heterocyst	Number of heterocysts	Position of heterocysts	Shape of Basal heterocyst
73.	Equal	Single, rarely several	Basal, rarely intercalary	Spherical or flattened
74.	Equal or heterocyst narrower	Single	Basal	Hemispherical compressed
75.	Equal or heterocyst wider	-	-	Compressed
76.	Equal	Several	Basal and few intercalary	Varied
77.	Equal	-	Basal	-
78.	-	Usually 2	Basal	-

Number	Shape of Sheath	Texture of Sheath	Width of Sheath	Colour of Sheath
73.	-	-	Thick 1.5 µm	Colourless and yellow at base
74.	-	Papery and firm	Thin 2.5 µm	Colourless
75.	Truncate	-	Wide < 4.5 µm	Colourless
76.	-	Gelatinous	Thick 4.5 µm	Yellow-brown
77.	Close, diffluent	-	Thin	Colourless
78.	-	Gelatinous	Thick	Colourless

Number	Sheath lamellations	Sheath lacerations	Spores	Habitat
73.	-	-	-	Geysir
74.	Present	Slightly, on outside	-	Mollusc shells and later, ponds
75.	-	-	-	Shallow river water
76.	Absent	-	-	Upper tidal pools, on rocks and lagae
77.	-	-	-	In water tank
78.	-	-	1-4, separated by heterocysts	Pond

Number	Organism	Physiognomic Form	Colony Height	Colonies Confluent or not
79.	R. aquatica	Spherical	2 mm high	-
80.	R. atra	Spherical or hemispherical	4 mm high	Isolated or confluent
81.	R. australis	Hemispherical, globular later	20 mm	Confluent
82.	R. beccariana	Hemispherical cushion	1-3.5 mm	Sometimes confluent
83.	R. biasolettiana	Hemispherical or expanded cushion	2-8 mm	Sometimes confluent
84.	R. borealis	Spherical or hemispherical	0.5 mm	Sometimes
85.	R. bornetiana	Spherical or hemispherical	1-10 mm	Confluent
86.	R. bullata	Expanded, lobed and hollow	60 mm	Confluent
87.	R. dura	Hemispherical	0.5-5 mm	Not confluent
88.	R. globiceps	Spherical or hemispherical	1.5-3 mm	Not confluent
89.	R. haematites	Hemispherical, later extending to form crust	30 mm	Confluent
90.	R. hansgiri	Expanded, flat and hollow. Nostocoid	-	-
91.	R. mamillata	Extensive cushion	0.5-1 mm	Confluent
92.	R. manginii	Spherical or sub-spherical	0.5-6 mm	Not confluent
93.	R. mesenterica	Expanded, lobed and hollow	40 mm	Confluent
94.	R. minutula	Spherical or hemispherical	5 mm	Confluent
95.	R. nitida	Spherical, expanded and hollow	30 mm	Confluent

Number	Colony Hollow or Solid	Calcifications	Distribution of Calcium Carbonate	Texture
79.	-	Absent	Not applicable	Soft
80.	Solid	Present	Scattered	Firm
81.	-	Absent	Not applicable	Soft
82.	-	Sometimes present	-	Firm
83.	-	Present	-	Soft or firm
84.	-	Present	-	Soft
85.	Solid	Sometimes present	-	Soft or firm
86.	Hollow	Absent	Not applicable	-
87.	-	Sometimes	-	Hard
88.	-	Absent	Not applicable	Soft or firm
89.	-	Present	Zoned	Firm
90.	Hollow	-	-	Gelatinous
91.	-	Present	Scattered	Firm
92.	-	Absent	Not applicable	Soft
93.	Hollow	Absent	Not applicable	Firm
94.	-	Sometimes	Centrally arranged	Soft
95.	-	Absent	Not applicable	Soft

Number	Arrangement of Trichomes	Tapering	Hair	Shape of Basal Cell
79.	Radial	Yes	Long and thin	Longer than broad
80.	Radial	Assumed	Thin	As long as broad barrel-shaped
81.	Radial	Narrow near base, widening and then tapering	Present	As long as broad or longer
82.	Radial	Present	Long and bent	Longer than broad
83.	Radial	Present	Long and bent	Shorter than broad or squarish
84.	Radial	Present	Present	Shorter than broad
85.	Radial	Assumed	Long when present	½-5 times longer than broad
86.	Radial	Abrupt taper	Present	As long as broad
87.	Radial	Present	Long and narrow	Squarish
88.	Radial	Present	Present	1½-4 times longer than broad
89.	Radial, becoming parellel when older	Assumed	Long	Twice as long as broad
90.	Horizontal, intricate, curved	Tapered at both ends	Present	Rectangual or sub-quadrate
91.	Radial	Present	Long	Shorter than broad or squarish
92.	Radial	Present	Long and thin	Slightly longer than broad
93.	Parellel	Abrupt taper	Present	As long as broad
94.	Radial	Present	Thick, with short cells	As long as broad
95.	Radial	Assumed	Long 3-4	3-4 times longer than broad

Number	Cell Width	Base of Trichome Swollen or Not	Cell Indentations	Width of Basal Compared to Heterocyst
79.	7-9 µm	-	-	Heterocyst wider
80.	2.5-8 µm	-	-	Heterocyst wider
81.	3 µm widening to 5-7.5 µm	-	-	Heterocyst much wider
82.	3-7 µm	-	-	Equal
83.	5-9 µm	-	-	Equal
84.	4 µm	No	-	Equal
85.	4-(16) µm	-	-	Heterocyst usually wider, 6-8 µm
86.	5-8-(10) µm	No	No	Equal
87.	4-9 µm	-	-	Basal cell slightly wider
88.	4.8-6 µm	-	-	Heterocyst wider
89.	4-7 µm	-	-	Heterocyst narrower or equal
90.	6 µm at centre 2-4 µm at ends	-	-	Equal or heterocyst slightly wider
91.	4-5 µm	-	-	Heterocyst wider
92.	3-5 µm	-	-	Equal
93.	7-12 µm	-	-	Heterocyst slightly wider
94.	9-12.5 µm	-	-	Equal
95.	2-5 µm	-	-	Heterocyst wider (may be narrower or equal)

Number	Number of Heterocysts	Position of Heterocysts	Shape of Basal Heterocyst	Shape of Sheath
79.	Single, basal	Basal	Spherical	-
80.	Single	Basal	Spherical	Diverging
81.	Usually single	Basal, rarely intercalary	Broader than long	-
82.	Single	Basal	Longer than broad, conical	-
83.	1-3	Basal, rarely intercalary	Globular, longer or shorter than broad	Funnel-shaped
84.	-	Basal	Spherical	Tapered
85.	Single	Basal	Depressed sphere or ellipsoidal	Funnel-shaped
86.	Single	Basal	Globose	Diverging in upper parts
87.	Single	Basal	Shorter than broad broad	Close
88.	1-2	Basal	Spherical	-
89.	-	Basal	Spherical or longer than broad	Funnel-shaped or narrow
90.	1-2 basal Seldom intercalary	Basal and seldom intercalary	-	Close
91.	-	-	-	Funnel-shaped
92.	Single or 2-4 together		Shorter than broad	Funnel-shaped
93.	Single	Basal	Longer than broad	Diverging in upper parts
94.	Single	Basal	Longer than broad, conical	Funnel-shaped
95.	1-2	Basal	Spherical or longer than broad	Diverging in upper parts

Number	Texture of Sheath	Width of Sheath	Colour of Sheath	Sheath Lamellations
79.	-	Thin	Colourless	Absent
80.	-	-	Colourless or pale yellow	Present
81.	-	Indistinct	Colourless or pale	Present
82.	-	Thin	Colourless, yellow or brown	Indistinct
83.	-	5-20 µm	Colourless or yellow-brown	Present
84.	-	-	Colourless	Absent
85.	-	2-20 µm	Colourless or yellow	-
86.	-	Thin	Colourless or yellow	Absent
87.	-	Thin	Hyaline	Absent
88.	-	Thick	Colourless	Absent
89.	-	-	Colourless or brown	Present
90.	-	Thin	Colourless or pale	-
91.	-	-	Colourless or pale	Present
92.	-	-	Hyalin Hyaline or brownish	Delicately lamellated
93.	-	Indistinct	Colourless or pale	-
94.	-	Wide (15 µm)	Colourless or brown	Present
95.	-	Indistinct	Colourless or yellow	Present

Number	Sheath Lacerations	Spores	Habitat
79.	-	Presumed absent	Freshwater
80.	Outer part lacerated	"	Marine, high water zone
81.	Present	"	Marine
82.	Lacerated at top	"	Freshwater
83.	Present	"	Freshwater or marine
84.	Lacerated when old	"	Freshwater
85.	-	"	Marine
86.	Absent	"	Marine
87.	Lacerated at top	"	Freshwater
88.	-	"	Freshwater
89.	-	"	Freshwater
90.	-	"	Freshwater
91.	-	"	Marine
92.	-	"	Freshwater
93.	Lacerated at top	"	Marine
94.	Present	"	Freshwater
95.	Often lacerated at top	"	Marine

Number	Organism	Physiognomic Form	Colony Height	Colonies Confluent or Not
96.	R. peguana	Expanded	-	-
97.	R. planctonica	Small colonies	0.5 mm	-
98.	R. polyotis	Spherical or hemispherical	2-30 mm	Confluent
99.	R. rufescens	Crust or hemispherical	15 mm	Confluent
100.	R. viellardi	Irregular deposits	10 mm	-

Number	Colony Hollow or Solid	Calcification	Distribution of Calcium Carbonate	Texture
96.	-	-	Not applicable	Gelatinous
97.	-	Absent	Not applicable	Soft
98.	Hollow	Absent	Not applicable	Soft
99.	-	Present	Central	Hard
100	-	-	Not applicable	Gelatinous

Number	Arrangement of Trichomes	Tapering	Hair	Shape of Basal Cell
96.	-	Accuminate	-	Twice as long as broad
97.	Radial	Assumed	Long	Squarish
98.	Radial	Present	Thick	Twice as long as broad, or squarish
99.	Radial	Assumed	Small and thick	Squarish
100.	-	Assumed	Present	Shorter than broad or barrel-shaped.

Number	Cell Width	Base of Trichome Swollen or Not	Cell Indentations	Width of Basal Cell Compared to Heterocyst
96.	7.5 μm	-	-	-
97.	4.6-5.8 μm	-	-	Heterocyst wider
98.	4.5-13.5 μm	No	Some	Equal
99.	8-12 μm	-	-	Equal
100.	7-9 μm	-	-	-

Number	Number of Heterocysts	Position of Heterocysts	Shape of Basal Heterocyst	Shape of Sheath
96.	-	-	-	-
97.	Single	Basal	Spherical or longer than broad	Close
98.	Single	Basal	Spherical, longer or shorter than broad	Funnel-shaped
99.	Single	Basal	Spherical or longer than broad	Funnel-shaped
100.	-	-	-	Funnel-shaped

Number	Texture of Sheath	Width of Sheath	Colour of Sheath	Sheath Lamellations
96.	-	-	-	-
97.	-	Narrow	Colourless	-
98.	-	Wide	Colourless or yellow-brown	Present
99.	-	Wide	Colourless or brown	Present
100.	-	Thick	Colourless or yellow	Present

Number	Sheath Lacerations	Spores	Habitat
96.	-	-	-
97.	-	-	-
98.	Present	-	Marine
99.	-	-	Freshwater
100.	Present	-	Soil (Freshwater)

APPENDIX III

Literature related to the distribution of Rivulariaceae

organism	author	date	habitat
Amphithrix janthina	Almodovar L.R.	1963	Puerto Rico
	Drouet F.	1938	Brazil, lake outlet
	Frémy P.	1924	France
	Hirsch A. & Palmer C.M.	1958	Ohio river, treatment filters
	Patrick R.	1961	East U.S.A. river
Calothrix adscendens	Almodovar L.R.	1963	Puerto Rico
C. aeruginea	Dutein F.	1962	Basin d'Arcachon
	Rathsack-Kunzenbach R.	1961	Östsee
C. anomala	Mitra A.K.	1951	Indian soil
C. braunii	Agarkar D.S.	1967	Pradesh, dripping rocks
	Borge O.	1921	Tåkernsees
	Cedercreutz C.	1933	Äland
	Drouet F.	1938	Brazil
	Renaut J. & Sasson A.	1970	Maroc
	Setchell W.A. & Gardner N.L.	1903	N.W. America
	Yoneda Y.	1952	Japan, thermal
C. breviarticulata	Neel J.K.	1968	Headwaters of limestone stream
C. brevissima	Bhashyakarla R.C.	1937	Orissa, India, on other algae
	"	1938	Madras, epiphyte
	Fjerdingstad E.	1965	French Alps
	Rana B.C. et al.	1971	
	West G.S.	1907	
	Yoneda Y.	1952	Japan, thermal
C. clavatoides	Kamat N.D.	1968	Bombay, embedded in Gloeotrichia

C. confervicola	Dutein F.	1962	Basin d'Arcachon
	Norton T.A.	1970	Co. Wexford, Ireland, eulittoral
	Webber E.E.	1967	Massachusetts salt-marsh
C. contarenii	Dutein F.	1962	Basin d'Arcachon
	Pankow H., Festerling E.& Festerling H.	1971	Östsee
C. crustacea	Cribb A.B.	1966	Great Barrier Reef
	Diaz-Piferrer M.	1962	Puerto Rico, marine
	Dutein F.	1962	Basin d'Arcachon
	Lawson G.W.	1960	Ghanian coast
	Setchell W.A.	1903	Brackish lagoon
	Webber E.E.	1967	Massachusetts salt marsh
	Whitton B.A.	1968	Sierra Leone
C. consociata	Setchell W.A. & Gardner N.L.	1903	Washington saltmarsh
C. desertica	Schwabe G.H.	1960	Arid ground
C. elenkinii	Bhashyakarla R.C.	1938	Madras, on floating log at edge of pond
C. epiphytica	Claus G.	1962	Hohle, standing water
	Howland L.J.	1931	Herefordshire pond
C. fasciculata	Pankow H., Festerling E. & Festerling H.	1971	Östsee
C. fusca	Bhashyakarla R.C.	1938	Orissa, India
	Borge O.	1921, 1923 & 1930	Sweden
	Cedercreutz C.	1933	Åland
	Fjerdingstad E.	1964	
	Golubić S.	1967	Titisees
	Kawecka B.	1971	Tatra mountains
	Prasad B.N. & Srivastava P.N.	1965	Himalayan hot spring
	Setchell W.A. & Gardner N.L.	1903	

C. fusca con.	Starmach K.	1958	Tatra mountains
	Wade W.E.	1949	Michigan Lake
	Yoneda Y.	1952	Japan, thermal
C. fusco-violacea	Serpette M.	1958	Mediterranean
C. galpinii	Cholonky K. & Pfarekuche K.	1968	Sudafrika
C. ibitinoensis	Yoneda Y.	1952	Japan, thermal
C. indica	Agarkar D.S.	1967	Pradesh, on submerged plants
C. juliana	Almodovar L.R.	1963	Puerto Rico
	Borge O.	1921	Täkernsees
	Patrick R.	1961	East U.S.A.
C. karnatakensis	Kamat N.D.	1968	Bombay
C. kossinskajae	Kullberg R.G.	1971	W. Montana, thermal
C. kuntzei	Yoneda Y.	1952	Japan,thermal
C. linearis	Bhasyakarla R.C.	1937	Orissa, India
C. marchica	"	"	" "
C. membranacea	Mitra A.K.	1951	Indian soil
C. parasitica	Rathsack-Künzenbach R.	1961	Östsee
C. parietina	Almodovar L.R.	1963	Puerto Rico
	Borge O.	1930	Sweden
	Cedercreutz C.	1933	Äland
	Drouet F.	1938	Brazil
	Dutein F.	1962	Basin d'Arcachon
	Fjerdingstad E.	1964	
	"	1965	French Alps
	Fritsch F.E.	1931	Central European lake
	Godward M.	1937	English lake, spray zone
	Golubić S.	1967	Titisees
	Heywood R.B.	1972	Anarctic, saline
	Hirsch A. & Palmer C.M.	1958	Hamiton Co, Ohio

C. parietina con	King L.J.	1943	Wayne Co. Indiana
	Lawson G.W.	1960	Ghanian coast
	Marchesoni V.	1939	Italy
	Patrick R.	1961	East U.S.A. river
	Setchell W.A. & Gardner N.L.	1903	
	Wade W.E.	1949	Michigan Lake
	Yoneda Y.	1952	Japan, thermal
C. pilosa	Cribb A.B.	1966	Great Barrier Reef
	Drouet F.	1938	Brazil
	Khan K.R.	1969	Oahu
C. pulvinata	Priou M.L. & Serpette M.	1954	Brittany
	Setchell W.A. & Gardner N.L.	1903	
C. scopulorum	Khan K.R.	1969	Oahu
	Rathsack-Künzenbach R.	1961	Ostsee
	Setchell W.A. & Gardner N.L.	1903	
	Stewart W.D.P.	1962	Scottish coast
C. scytonemicola	Agarkar D.S.	1967	Pradesh
	Louis A., De Baeck W. & Podoor N.	1967	Dyle
C. sphaerospora	Pradesh B.N. & Srivastava P.N.	1965	Lucknow
C. stagnalis	Cedercreutz C.	1933	Åland
C. thermalis	Kullberg R.G.	1971	W. Montana, thermal
	Mann J.E. & Schlichtling H.E.Jr.	1967	Yellowstone U.S.A. thermal
	Yoneda Y.	1952	Japan thermal
C. turfacea	"	"	"
C. viguieri	Bhasyakarla R.C.	1936	Madras
Dichothrix baueriana	Almodovar L.R.	1963	Puerto Rico
	Borge O.	1921 & 1930	Täkernsees

D. baueriana con.	Gupta R.S.	1972	Rajasthan
	Nauwerck A.	1968	Swedish Lapland
	Schumacher G.J., Bellis V.J. & Whitford L.A.	1963	N. Carolina
D. baueriana var. crassa	Brook A.J.	1957	Scottish loch
	Godward M.	1937	English lake
D. calderia	Yoneda Y.	1952	Japan, thermal
D. compacta	Borge O.	1930	Sweden
	Cedercreutz C.	1933	Åland
	Golubić S.	1967	Titisees
D. fusca	Agarkar D.S.	1967	Pradesh
D. horsfordii	Fjerdingstad E.	1964	
D. gypsophila	Agarkar D.S.	1967	Pradesh
	Almodovar L.R.	1963	Puerto Rico
	Cedercreutz C.	1933	Åland
	Dor I.	1970	Lake Tiberias
	Fjerdingstad E.	1964	
D. montana	Kullberg R.G.	1971	W. Montana, thermal
D. olivacea	Diaz-Piferrer M.	1962	Puerto Rico, intertidal pools
D. orsiniana	Bendre A.M. & Agarkar M.S.	1965	Bhopal, wet soil
	Godward M.	1937	Edge of calcareous lake
Gloeotrichia echinulata	Benson-Evans K., Fisk D., Pickup G. & Davies P.	1971	Slapton Ley
	Bhashyakarla R.C.	1938	Orissa, pond
	Cedercreutz C.	1933	Åland
	Griffiths B.M.	1926	Angelsey
	Lund J.W.G.	1965	
	Pieczynska E.	1965	Lake Mikoljki
	Reynolds C.S.	1971	White Mere, Shropshire
	Roelofs T.D. & Oglesby R.T.	1970	Green Lake Seattle

G. echinulata con.	Sinker C.A.	1962	N. Shropshire Meres
	Spondniewsk I.	1967	Poland, lake
	Stein J.R.	1960	Hubbel Pond
	Trond G.C. Jr.	1961	U.S.A. paddy fields
	Willén T.	1961	S. Sweden, lake
G. ghosei	Agarkar D.S.	1967	Pond
	Singh S.B.	1972	Gorakhpur, pond
G. intermedia	Bhashyakarla R.C.	1936	Rainwater pools
	Cedercreutz C.	1933	Äland
G. karnatakensis	Kamat N.D.	1968	Paddy fields
G. natans	Agarkar D.S.	1967	Pradesh, lake
	Bhashyakarla R.C.	1936	United Province, Indi
	"	1938	Madras
	Cedercreutz C.	1933	Äland
	Kamat N.D.	1968	Bombay
	Singh S.B.	1972	Gorakhpur
	Sbornik & Ceophwk	1963	Prague, lake
	Trond G.S.	1961	Araneta Campus U.S.A.
	Yoneda Y.	1952	Japan, thermal
G. pisum	Cedercretz C.	1933	Äland
	Gibbons D.S. & Whitton B.A.	1966	Co. Durham
	Leentuarur P.	1963	Lake, on Chara
	Singh S.B.	1972	Gorakhpur, pond
	Wade W.E.	1949	Michigan, lake
	Young O.W.	1945	Michigan, lake
G. pilgeri	Bendre A.M. & Agarkar M.S.	1965	Bhopal
	Kamat N.D.	1968	Bombay
	Prasad B.N.	1962	Uttar Pradesh

G. raciborskii	Bhashyakarla R.C.	1937	India
	"	1938	Orissa, in puddle
	"	"	Madras, in puddle
	Bendre A.K. & Agarkar M.S.	1965	Bhopal
	Kamat N.D.	1961	Bombay, paddy fields
	Munawar M.	1970	Hyderbad
	Prasad B.N.	1962	Uttar Pradesh
Hammatoidea normani	Golubić S.	1967	Titisees
	Kawecka B.	1971	Tatra mountains
	Starmach K.	1927	Tatra mountains
H. multispora	Kullberg R.G.	1971	W. Montana, thermal
H. olivacea	"	"	"
	Yoneda Y.	1952	Japan, thermal
Homoeothrix crustacea	Kann E.	1966	Austria
	Starmach K.	1966	River Raba
H. fusca	Golubić S.	1967	Titisees
	Starmach K.	1968	Poland
H. janthina	Backhaus D.	1967, 1968 & 1969	Headwaters of Donau and Danube
	Golubić S.	1967	Titisees
	Starmach K.	1959	
H. juliana	Kann E.	1966	Austria
H. thermalis	"	1969	
	Yonedo Y,	1952	Japan, thermal
H. varians	Kann E.	1966 & 1969	Austria
Leptochaete crustacea	Starmach K.	1927	Tatra mountains
L. stagnalis	Borge O.	1923	Sweden
Polythrix sp.	Friedmann I.	1956	

Rivularia atra	Cribb A.B.	1966	Great Barrier Reef
	Dutein F.	1962	Basin d'Arcachon
	Gamulin-Brida H., Gioccone G. & Golubić S.	1967	Heligoland
	Norton T.A.	1970	Ireland
	Priou M.L. & Serpette M.	1954	Brittany
	Rathsack R.	1966	
	Webber E.E.	1967	Massachusetts salt-marsh
R. aquatica	Agarkar D.S.	1967	Pradesh
	Bendre A.M. & Agarkar M.S.	1965	Bhopal
R. beccariana	West W.	1912	Clare Is., Ireland
R. biasolettiana	Cedercreutz C.	1933	Åland
	Fritsch F.E.	1931	European lake
	Hansen K.	1967	Greenland, lake
	Hughes M.K. & Whitton B.A.	1972	N.E. England
	Kann E.	1961	Lake
	Setchell W.A. & Gardner N.L.	1903	
	Yoneda Y.	1952	Japan, thermal
R. borealis	Forster J.W. Jr. & Schlichting H.E. Jr.	1965	Lake Operongo
	Hansen K.	1967	Greenland, lake
R. bornetiana	Setchell W.A.	1895	New England
R. bullata	Norton T.A.	1970	Ireland
	Quillet M. & Lestang-Laisne G.	1967	Lichen
	Rees T.K.	1939	Kennack sands
R. dura	Young O.W.	1945	Michigan, lake
R. globiceps	Bhashyakarla R.C.	1938	Madras
	Kattick F. & Gerloff J.	1965	S.W. Africa

R. haematites	Carter N.	1922	New Caledonia
	Fritsch F.E.	1931	Lake
	Picken L.E.R.	1936	Yugoslavia, limestone spring
	Stein J.R.	1960	Hubbel pond
	West W.	1912	Clare Is. Ireland
R. minutula	Benson-Evans K., Fisk D., Pickup G. & Davies P.	1971	Slapton Ley
	Borge O.	1921	Täkernsees
	Stein J.R.	1960	Hubbel Pond
R. nitida	Dutein F.	1962	Basin d'Arcachon
	Priou M.L. & Serpette M.	1954	Britanny
	Rathsack R.	1966	
	Rathsack-Künzenbach R.	1961	Östsee
	Setchell W.A. & Gardner N.L.	1903	
	Webber E.E.	1967	Massachusetts salt-marsh
	West W.	1912	Clare Is. Ireland
R. polyotis	Dutein F.	1962	Basin d'Arcachon
	Starmach K.	1927	Poland
R. rufescens	Fjerdingstad E.	1964	
Tapinothrix mucicola	Borge O.	1923	Sweden

APPENDIX IV

Field data and trichome descriptions

of *Rivularia*.

Guide to computer records of

Rivularia samples

code number	stream and reach	location	grid reference	collection date
10001		Slapestone Sike	NY 816304	1.4.71
10002		Slapestone Sike	NY 815304	1.4.71
10003		Slapestone Sike	NY 814304	1.4.71
10011		Sunbiggin flush	NY 672077	17.4.71
10012		"	"	"
10013		"	"	"
10014		Sunbiggin flush	NY 672078	17.4.71
10015		"	NY 671077	"
10016		"	"	"
10020		Loch a' Chapuill	NC 096181	21.4.71
10021			NC 105178	21.4.71
10040		Cwm Nofydd	ST 147837	6.5.72
10041		"	"	"
10042		"	"	"
10043		"	"	"
10044		"	"	"
10045		"	"	"
10053		Croft	NZ 102108	7.6.71
10056		Nameless Sike	NY 817296	11.6.71
10071		Kishorn	NG 842432	24.8.71
10085		Red Sike	NY 816294	28.9.71
10086		Malham Tarn, north shore	SD 897672	16.10.71
10087		"	"	"
10088		"	"	"
10089		Gordale Beck, ford	SD 912656	16.10.71
10090		"	"	"
10091		"	"	"
10092		Gordale Beck, side flush	SD 910655	16.10.71
10093		"	"	"
10094		"	"	"
10099		Bowlees	NY 907284	2.11.71

code number	stream and reach	location	grid reference	collection date
10100		Tulach hill	NN 869643	23.9.72
10101		"	NN 869644	"
10102		"	NN 869645	"
10104		Skiag Bridge	NC 236250	24.9.72
10105		"	NC 237251	"
10106		"	NC 238256	"
10112		side flush, Allt a Chalda Mor	NC 246237	24.9.72
10113		"	"	"
10115		stream flowing into Allt a Chalda Mor	NC 247235	"
10116		"	"	"
10120		Allt a Glaic Moire	NC 263214	"
10125		stream behind Inchnadamph Hotel	NC 255217	"
10150		Barras	NY 847122	19.6.73

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
10471	NY816304	10001	1	7	3	3	3	3	2	1	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	2	3	1	5	2	4	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	330	1290	9.0	1	2	2	2	1	1	3	9.0	10.5	1	2	4.5	2	2	2	2	2
2	1	3	240	420	9.0	1	2	2	3	1	1	1	11.0	11.0	3	2	1.5	3	3	2	2	2
3	5	1	0	600	12.0	1	2	2	2	1	1	1	12.0	12.0	3	2	1.5	4	3	2	2	2
4	5	1	0	450	9.0	1	2	2	2	1	1	3	9.0	10.5	3	2	1.5	4	3	2	2	2
5	1	3	200	350	5.0	2	2	2	3	1	1	1	10.5	10.5	3	2	3.0	2	2	2	2	2
6	5	1	0	690	9.0	1	2	2	2	1	1	1	9.0	9.0	3	2	3.0	3	3	2	2	2
7	1	3	300	1150	9.0	1	2	2	3	1	1	2	12.0	10.5	1	2	3.0	2	2	2	2	2
8	1	3	300	450	9.0	2	2	2	2	1	1	1	9.0	9.0	3	2	3.0	3	3	2	2	2
9	1	3	200	410	9.0	1	2	2	2	1	1	2	9.0	6.0	3	2	4.5	2	3	2	2	2
10	5	1	0	420	10.5	3	2	2	3	1	1	2	12.0	11.0	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
10471	NY815304	10002	1	7	3	3	3	3	2	1	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	2	3	1	5	2	2	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	180	360	4.0	2	2	2	3	1	1	1	12.0	12.0	1	2	2.0	3	3	2	2	2
2	3	1	0	45	9.0	1	2	2	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
3	1	3	50	150	9.0	1	2	2	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
4	5	1	0	360	9.0	1	2	2	3	1	1	3	12.0	15.0	1	2	3.0	3	3	2	2	2
5	1	3	300	660	12.0	1	2	2	3	1	1	2	15.0	12.0	3	2	1.5	4	3	2	2	2
6	1	3	150	210	6.0	2	2	2	2	1	1	3	6.0	9.0	1	2	4.0	2	2	2	2	2
7	1	3	30	160	6.0	2	2	2	2	1	1	3	6.0	9.0	1	2	4.0	2	2	2	2	2
8	1	3	30	160	5.0	1	2	2	3	1	1	3	6.0	12.0	1	2	4.0	2	3	2	2	2
9	1	3	150	270	9.0	1	2	2	1	1	1	3	6.0	6.0	1	2	3.0	3	3	2	2	2
10	1	3	150	630	6.0	1	2	2	9	9	9	9	0.0	0.0	1	2	4.5	2	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
10471	NY814304	10003	1	7	3	3	3	3	2	1	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	2	3	3	5	2	2	5	3

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	300	540	9.0	1	2	2	3	1	1	2	13.0	10.5	3	2	1.5	4	3	2	2	2
2	9	1	0	75	6.0	2	2	2	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
3	5	1	0	1200	10.5	1	2	2	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
4	1	3	210	510	9.0	1	2	2	2	1	1	1	9.0	9.0	3	2	1.5	4	3	2	2	2
5	1	3	180	330	7.5	1	2	2	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
6	9	1	0	240	9.0	1	2	2	3	1	1	1	12.0	12.0	3	2	1.5	4	3	2	2	2
7	1	3	45	195	9.0	1	2	2	2	1	1	1	9.0	9.0	3	2	1.5	4	3	2	2	2
8	9	1	0	75	12.0	1	2	2	1	1	1	1	9.0	6.0	2	2	3.0	3	3	2	2	2
9	1	3	150	500	6.0	1	2	2	2	1	1	3	6.0	9.0	3	2	1.5	4	3	2	2	2
10	1	3	120	210	6.0	3	2	2	2	1	1	1	6.0	6.0	3	2	1.5	4	3	2	2	2

CATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
17C471	NY672C77	10011	1	7	3	3	3	4	1	1	1	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	2	1	1	2	3	1	1	3	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	5	1	C	60C	9.C	0	2	0	3	1	1	1	10.5	10.5	1	2	3.0	2	1	2	2	2
2	5	1	0	600	12.0	0	2	0	2	1	1	3	12.0	10.5	3	2	4.5	2	1	2	2	2
3	1	3	150	750	9.0	0	2	0	3	1	1	2	12.0	9.C	3	2	3.0	2	3	2	2	2
4	9	1	0	36	12.C	0	2	C	9	9	9	9	0.0	0.C	3	2	1.5	4	3	2	2	2
5	1	3	100	51C	9.0	0	2	0	3	1	1	2	15.0	10.5	1	2	1.5	4	3	2	2	2
6	9	1	0	39	9.C	0	2	0	9	9	9	9	0.0	0.C	3	2	1.5	4	3	2	2	2
7	1	3	150	960	9.0	0	2	0	2	1	1	3	9.0	10.5	3	2	1.5	3	3	2	2	2
8	5	1	0	240	9.C	0	2	0	9	9	9	9	0.0	C.C	3	2	3.0	3	3	2	2	2
9	1	3	150	60C	9.C	0	2	0	3	1	1	2	10.5	9.0	1	2	3.0	3	3	2	2	2
10	5	1	0	660	6.0	0	2	0	9	9	9	9	0.0	0.C	3	2	3.0	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
170471	NY672077	10012	1	7	3	3	3	4	1	1	1	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	2	1	1	2	3	1	1	3	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	9	1	0	90	6.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
2	9	1	0	300	6.0	0	2	0	9	9	9	9	0.0	0.0	3	2	3.0	3	3	2	2	2
3	5	1	0	630	9.0	0	2	0	2	1	1	1	9.0	9.0	3	2	3.0	4	3	2	2	2
4	1	3	120	660	9.0	0	2	0	2	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
5	1	3	180	480	9.0	0	2	0	3	1	1	2	12.0	10.5	3	2	1.5	4	3	2	2	2
6	9	1	0	39	9.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
7	1	3	210	510	9.0	0	2	0	3	1	1	3	10.5	12.0	3	2	1.5	4	3	2	2	2
8	9	1	0	360	10.5	0	2	0	9	9	9	9	0.0	0.0	3	2	3.0	3	3	2	2	2
9	1	3	180	540	9.0	0	2	0	2	1	1	1	9.0	9.0	3	2	1.5	4	3	2	2	2
10	1	3	90	360	9.0	0	2	0	3	1	1	2	12.0	10.5	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
170471	NY672077	10013	1	7	3	3	3	4	1	1	1	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	2	1	1	2	3	1	1	3	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	9	1	0	45	9.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	2	2	2	2	2
2	5	1	0	480	12.0	0	2	0	2	1	1	3	12.0	15.0	1	2	3.0	2	2	2	2	2
3	1	3	120	390	9.0	0	2	0	3	1	1	2	10.5	9.0	1	2	3.0	3	3	2	2	2
4	5	1	0	300	9.0	0	2	0	9	9	9	9	0.0	0.0	3	2	3.0	4	3	2	2	2
5	1	3	150	540	12.0	0	2	0	2	1	1	2	12.0	10.5	3	2	1.5	4	3	2	2	2
6	1	3	180	480	10.5	0	2	0	3	1	1	2	12.0	10.5	3	2	1.5	4	3	2	2	2
7	9	1	0	45	12.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
8	5	1	0	420	9.0	0	2	0	3	1	1	3	10.5	12.0	3	2	3.0	4	3	2	2	2
9	1	3	210	600	9.0	0	2	0	3	1	1	1	10.5	10.5	3	2	1.5	4	3	2	2	2
10	5	1	0	600	12.0	0	2	0	2	1	1	2	12.0	10.5	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
170471	NY668C77	10014	1	7	3	3	3	1	9	5	1	1	9	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	6	1	1	2	3	1	3	1	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	480	780	9.0	0	0	0	3	1	1	2	10.5	9.C	1	2	3.0	4	2	2	2	2
2	1	3	540	840	6.0	0	0	0	3	1	1	2	9.0	7.5	1	2	6.0	2	1	2	2	2
3	9	1	0	90	6.0	0	0	0	9	9	9	9	C.C	0.C	3	2	1.5	4	3	2	2	2
4	5	1	0	780	6.0	0	0	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
5	1	3	120	360	9.0	0	0	0	3	1	1	2	10.5	9.0	1	2	3.0	3	2	2	2	2
6	1	3	510	900	9.C	C	0	0	3	1	1	2	10.5	9.0	1	2	3.0	4	2	2	2	2
7	1	3	420	870	6.C	0	0	C	3	1	1	2	9.0	7.5	1	2	3.0	4	2	2	2	2
8	1	1	0	6C0	6.C	0	0	0	3	1	1	2	7.5	6.0	1	2	6.0	2	1	2	2	2
9	1	3	300	660	9.0	0	0	0	9	9	9	9	0.0	0.C	3	2	3.0	4	2	2	2	2
10	1	3	210	51C	6.0	0	0	0	9	9	9	9	0.0	0.0	3	2	3.0	4	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	F	F	G	H	I	J	K
170471	NY668077	10015	1	7	3	3	3	1	9	2	1	3	3	1

MACROCHARACTERS

A	B	C	D	E	F	G	H	I	J	K	L
2	2	1	1	1	2	1	1	3	3	2	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	9	1	0	240	9.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	3	3	2	2	2
2	9	1	0	81	9.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	3	3	2	2	2
3	1	3	120	240	9.0	0	0	0	3	1	1	2	10.5	10.5	1	0	3.0	2	1	2	2	2
4	1	3	60	300	7.5	0	0	0	2	1	1	2	7.5	6.0	3	0	1.5	3	3	2	2	2
5	9	1	0	90	9.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
6	9	1	0	60	9.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
7	5	1	0	300	9.0	0	0	0	2	1	1	2	9.0	7.5	3	0	1.5	3	3	2	2	2
8	9	1	0	270	9.0	0	0	0	9	9	9	9	0.0	0.0	3	0	3.0	3	3	2	2	2
9	1	1	0	240	9.0	0	0	0	3	1	1	3	10.5	12.5	3	0	1.5	3	3	2	2	2
10	9	1	0	180	9.0	0	0	0	2	1	1	3	9.0	10.5	3	0	1.5	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	F	F	G	H	I	J	K
170471	NY668077	10016	1	7	3	3	3	4	9	1	1	3	1	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	3	1	1	2	4	4	4

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	5	1	0	490	6.0	0	0	0	3	1	1	3	7.5	9.0	3	1	1.5	4	3	2	2	2
2	5	1	0	255	9.0	0	0	0	3	1	1	3	10.5	12.0	3	1	1.5	4	3	2	2	2
3	1	3	60	270	9.0	0	0	0	3	1	1	3	12.0	15.0	3	1	1.5	4	3	2	2	2
4	9	1	0	120	7.5	0	0	0	9	9	9	9	0.0	0.0	3	1	1.5	4	3	2	2	2
5	5	1	0	750	9.0	0	0	0	3	1	1	3	10.5	12.0	3	1	1.5	4	3	2	2	2
6	5	1	0	900	9.0	0	0	0	2	1	1	3	9.0	10.5	1	1	3.0	2	2	2	2	2
7	1	3	330	630	6.0	0	0	0	2	1	1	3	6.0	9.0	1	1	3.0	2	2	2	2	2
8	5	1	0	540	9.0	0	0	0	3	1	1	3	10.5	12.0	3	1	1.5	4	3	2	2	2
9	1	3	240	720	9.0	0	0	0	3	1	1	3	10.5	15.0	3	1	1.5	4	3	2	2	2
10	1	3	120	390	7.5	0	0	0	3	1	1	3	10.5	12.0	3	1	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210471	NC096181	10020	2	7	2	2	0	5	2	4	3	4	0	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	2	1	3	9	3	5	0	1	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	1	0	400	8.0	0	0	0	3	1	1	1	9.0	9.0	3	0	1.5	4	3	2	2	2
2	1	1	0	120	8.0	0	0	0	3	1	1	2	10.5	9.0	3	0	1.5	4	3	2	2	2
3	9	1	0	150	8.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
4	1	1	0	160	12.0	0	0	0	3	1	1	2	15.0	12.0	3	0	3.0	2	3	2	2	2
5	1	1	0	150	8.0	0	0	0	3	1	1	2	12.0	10.5	3	0	1.5	3	3	2	2	2
6	1	3	300	720	9.0	0	0	0	3	1	1	1	12.0	12.0	3	0	3.0	2	3	2	2	2
7	1	3	300	450	7.5	0	0	0	3	1	1	2	10.5	9.0	3	0	3.0	2	3	2	2	2
8	1	3	300	450	10.5	0	0	0	3	1	1	2	15.0	12.0	3	0	1.5	3	3	2	2	2
9	1	3	600	750	7.5	0	0	0	3	1	1	2	10.5	9.0	3	0	1.5	4	3	2	2	2
10	1	1	0	150	9.0	0	0	0	3	1	1	2	12.0	10.5	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210471	NC105178	10021	2	7	2	2	0	5	2	4	3	4	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	2	1	3	9	3	5	0	1	3	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	1	0	165	12.0	0	0	0	3	1	1	1	15.0	15.0	1	0	3.0	2	1	2	2	2
2	5	1	0	200	8.0	0	0	0	3	1	1	1	10.5	10.5	1	0	3.0	2	1	2	2	2
3	1	1	0	150	8.0	0	0	0	3	1	1	2	12.0	10.5	1	0	4.5	2	1	2	2	2
4	1	1	0	300	8.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
5	1	1	0	240	8.0	0	0	0	3	1	1	1	9.0	9.0	3	0	1.5	4	3	2	2	2
6	1	3	60	270	9.0	0	0	0	3	1	1	1	10.5	10.5	1	0	3.0	2	1	2	2	2
7	1	1	0	210	6.0	0	0	0	3	1	1	2	9.0	7.5	1	0	4.5	2	1	2	2	2
8	1	1	0	180	6.0	0	0	0	3	1	1	2	9.0	7.5	1	0	3.0	2	1	2	2	2
9	1	3	90	360	6.0	0	0	0	3	1	1	1	7.5	7.5	1	0	3.0	2	1	2	2	2
10	9	1	0	600	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210571	ST147837	10040	1	7	3	3	7	5	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	2	2	2	3

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	150	900	3.0	0	0	0	3	1	1	3	9.0	10.5	1	0	3.0	2	3	2	2	2
2	1	9	0	45	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
3	1	3	45	300	6.0	0	0	0	3	1	1	2	7.5	6.0	3	0	1.5	3	3	2	2	2
4	1	3	150	330	4.5	0	0	0	3	1	1	2	7.5	6.0	3	0	1.5	4	3	2	2	2
5	5	1	0	450	4.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
6	5	1	0	900	7.5	0	0	0	9	9	9	9	0.0	0.0	1	0	3.0	2	3	2	2	2
7	1	1	0	210	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	3.0	3	3	2	2	2
8	1	3	120	300	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2
9	9	1	0	60	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
10	5	1	0	480	4.5	0	0	0	3	1	1	3	9.0	10.5	1	0	3.0	2	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210571	ST147837	10041	1	7	3	3	7	5	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	2	2	4	3

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	60	300	6.0	0	0	0	3	1	1	3	7.5	9.0	1	0	3.0	2	3	2	2	2
2	1	3	180	480	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2
3	9	1	0	90	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
4	1	3	120	360	4.5	0	0	0	3	1	1	3	6.0	7.5	3	0	3.0	3	3	2	2	2
5	5	1	0	720	4.5	0	0	0	9	9	9	9	0.0	0.0	3	0	3.0	3	3	2	2	2
6	5	1	0	360	6.0	0	0	0	3	1	1	3	7.5	9.0	1	0	30.0	2	3	2	2	2
7	1	3	90	180	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2
8	1	3	120	300	6.0	0	0	0	3	1	1	3	7.5	8.0	1	0	3.0	2	3	2	2	2
9	9	1	0	60	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
10	1	3	90	420	4.5	0	0	0	3	1	1	3	6.0	7.0	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210571	ST147837	10042	1	7	3	3	7	5	2	2	2	3	4	1

MACROCHARACTERS

A	B	C	D	E	F	G	H	I	J	K	L
2	3	1	1	1	2	1	1	2	4	2	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	150	900	4.5	0	0	0	3	1	1	3	6.0	7.5	1	0	3.0	3	3	2	2	2
2	1	3	120	720	6.0	0	0	0	3	1	1	3	7.5	9.0	1	0	3.0	2	3	2	2	2
3	1	3	90	300	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2
4	9	1	0	60	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
5	9	1	0	45	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
6	1	3	210	510	4.5	0	0	0	3	1	1	3	6.0	7.5	3	0	1.5	4	3	2	2	2
7	1	3	120	420	4.5	0	0	0	3	1	1	3	6.0	7.5	3	0	1.5	4	3	2	2	2
8	1	3	240	600	6.0	0	0	0	3	1	1	3	9.0	10.5	1	0	3.0	2	3	2	2	2
9	1	3	180	720	6.0	0	0	0	3	1	1	3	7.5	9.0	1	0	3.0	2	3	2	2	2
10	1	3	90	360	4.5	0	0	0	3	1	1	3	6.0	7.5	1	0	3.0	2	3	2	2	2

CATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210571	ST147837	10043	1	7	3	3	3	5	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	2	1	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	150	270	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	3.0	3	3	2	2	2
2	9	1	0	90	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
3	1	3	120	240	7.5	0	0	0	3	1	1	2	9.0	7.5	3	0	1.5	4	3	2	2	2
4	1	3	120	255	7.5	0	0	0	3	1	1	2	9.0	7.5	3	0	1.5	4	3	2	2	2
5	1	3	30	420	7.5	0	0	0	3	1	1	2	9.0	7.5	3	0	1.5	4	3	2	2	2
6	1	3	90	300	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	3.0	4	3	2	2	2
7	9	1	0	60	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
8	1	3	135	420	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
9	1	3	90	270	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2
10	1	3	180	420	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210571	ST147837	10044	1	7	3	3	3	5	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	2	1	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	180	360	7.5	0	0	0	3	1	1	3	9.0	10.5	3	0	3.0	3	3	2	2	2
2	1	3	75	270	7.5	0	0	0	3	9	9	9	0.0	0.0	3	0	3.0	2	3	2	2	2
3	3	1	0	90	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
4	1	3	120	300	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
5	1	3	135	330	7.5	0	0	0	2	1	1	3	7.5	9.0	3	0	3.0	4	3	2	2	2
6	1	3	60	360	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	1.5	4	3	2	2	2
7	9	1	0	75	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
8	1	3	30	420	7.5	0	0	0	2	1	1	3	7.5	9.0	3	0	3.0	3	3	2	2	2
9	1	3	75	360	7.5	0	0	0	2	1	1	3	7.5	9.0	3	0	3.0	4	3	2	2	2
10	9	1	0	45	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
210571	ST147837	10045	1	7	3	3	3	5	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	2	1	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	90	390	7.5	0	0	0	2	1	1	3	7.5	9.0	3	0	3.0	3	3	2	2	2
2	1	3	180	540	6.0	0	0	0	3	1	1	3	7.5	9.0	3	0	3.0	2	3	2	2	2
3	1	3	120	480	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	3.0	3	3	2	2	2
4	9	1	0	60	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
5	9	1	0	45	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
6	1	3	60	240	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
7	1	3	135	300	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
8	1	3	210	480	7.5	0	0	0	2	1	1	3	7.5	9.0	3	0	3.0	3	3	2	2	2
9	1	3	120	360	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	3.0	2	3	2	2	2
10	3	1	0	120	6.0	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
70671	NZ102108	10053	2	7	3	3	13	9	9	9	3	4	1	1

MACROCHARACTERS

A	B	C	D	E	F	G	H	I	J	K	L
2	3	2	1	1	3	3	5	1	4	9	2

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	15	450	9.0	0	0	0	2	1	1	1	9.0	9.0	1	0	3.0	2	2	2	2	2
2	1	3	900	1110	9.0	0	0	0	2	1	1	1	9.0	9.0	1	0	3.0	2	2	2	2	2
3	1	3	360	810	9.0	0	0	0	2	1	1	1	9.0	9.0	1	0	1.5	2	2	2	2	2
4	1	3	900	1240	9.0	0	0	0	2	1	1	1	9.0	9.0	1	0	1.5	2	2	2	2	2
5	1	3	600	960	9.0	0	0	0	2	1	1	1	9.0	9.0	1	0	1.5	2	2	2	2	2
6	1	3	120	480	9.0	0	0	0	2	1	1	3	9.0	12.0	1	0	1.5	2	2	2	2	2
7	1	3	300	630	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	2	3	2	2	2
8	1	3	210	250	6.0	0	0	0	2	1	1	1	6.0	6.0	1	0	1.5	2	2	2	2	2
9	1	1	165	165	10.5	0	0	0	1	1	1	2	9.0	6.0	1	0	3.0	2	2	2	2	2
10	1	1	150	1150	7.5	0	0	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
110671	NX817296	10056	1	7	3	3	1	1	2	4	2	2	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	3	1	1	1	1	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	600	810	9.0	1	2	2	2	1	1	3	9.0	10.5	1	2	3.0	2	3	2	2	2
2	1	1	0	1120	9.0	1	2	2	3	1	1	3	12.0	17.5	3	2	1.5	4	3	2	2	2
3	1	3	450	1350	10.5	1	2	2	3	1	1	3	12.0	18.0	3	2	1.5	3	3	2	2	2
4	1	3	90	390	12.0	2	2	2	2	1	1	1	12.0	12.0	1	2	3.0	4	3	2	2	2
5	1	3	60	330	9.0	1	2	2	3	1	1	2	10.5	10.5	3	2	1.5	4	3	2	2	2
6	9	1	0	60	6.0	2	2	3	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
7	5	1	0	900	9.0	2	2	3	3	1	1	3	12.0	18.0	3	2	3.0	3	3	2	2	2
8	1	1	0	240	9.0	1	2	2	3	1	1	1	10.5	10.5	3	2	1.5	4	3	2	2	2
9	1	3	90	300	9.0	1	2	2	3	1	1	1	10.5	10.5	3	2	1.5	4	3	2	2	2
10	1	3	300	540	10.5	1	2	2	2	1	1	3	12.0	15.0	3	2	1.5	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240871	NG842432	10071	1	7	2	2	3	5	1	1	2	1	1	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	2	1	1	2	1	1	1	0	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	1	0	150	6.0	1	2	2	3	1	1	2	7.5	6.0	1	0	4.5	1	2	1	2	2
2	1	1	0	300	6.0	1	1	2	3	1	1	3	7.5	9.0	1	1	4.5	1	2	1	2	2
3	1	3	30	330	9.0	1	2	2	2	1	1	2	9.0	6.0	1	0	4.5	1	2	1	2	2
4	1	3	60	180	6.0	2	2	2	3	1	1	2	9.0	6.0	1	0	3.0	1	2	1	2	2
5	1	1	0	360	9.0	1	2	2	3	1	1	2	12.0	7.5	1	0	3.0	2	2	1	2	2
6	1	3	90	240	6.0	1	2	2	2	1	1	3	6.0	7.5	1	0	4.5	1	2	1	2	2
7	9	1	0	60	4.5	2	2	3	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
8	1	1	0	180	6.0	1	2	2	3	1	1	3	7.5	9.0	1	0	3.0	1	2	1	2	2
9	1	3	60	180	6.0	1	2	2	3	1	1	2	9.0	6.0	1	0	3.0	2	2	1	2	2
10	1	1	0	240	9.0	1	2	2	2	1	1	2	9.0	7.5	1	0	3.0	2	2	1	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
280971	NY816294	10085	1	7	3	3	3	5	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	2	3	3	5	2	0	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	210	480	7.5	1	2	2	3	1	1	2	9.0	7.5	1	2	3.0	2	1	1	2	2
2	1	3	120	660	9.0	3	2	2	2	1	1	2	9.0	6.0	1	2	4.5	1	1	1	2	2
3	1	3	300	810	9.0	1	2	2	3	1	1	2	10.5	7.5	1	2	3.0	2	1	1	2	2
4	9	1	0	150	6.0	2	2	2	0	9	9	2	0.0	0.0	3	2	1.5	4	3	2	2	2
5	1	3	150	540	10.5	1	2	2	2	2	1	2	10.5	9.0	1	2	4.5	1	1	1	2	2
6	1	3	159	450	7.5	3	2	2	3	1	1	2	9.0	6.0	1	2	3.0	2	1	1	2	2
7	1	3	270	630	10.5	1	2	2	2	1	1	2	10.5	7.5	1	2	3.0	2	1	1	2	2
8	9	1	0	60	6.0	2	2	2	0	9	9	2	0.0	0.0	3	2	3.0	4	3	2	2	2
9	9	1	0	90	6.0	2	2	2	0	9	9	2	0.0	0.0	3	2	1.5	4	3	2	2	2
10	1	3	336	780	10.5	1	2	2	2	2	1	2	10.5	7.5	1	2	3.0	2	1	1	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD897672	10086	1	7	4	3	3	4	2	3	2	5	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	2	3	1	1	1	3	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	90	300	6.0	0	0	0	2	2	4	3	6.0	7.5	3	1	3.0	3	3	2	2	2
2	1	3	120	480	6.0	0	0	0	2	2	4	3	6.0	7.5	3	1	3.0	3	3	2	2	2
3	4	1	9	900	4.5	0	0	0	3	1	1	3	6.0	7.5	3	1	1.5	4	3	2	2	2
4	1	3	75	630	5.0	0	0	0	1	1	1	3	4.5	6.0	3	2	3.0	3	3	2	2	2
5	1	3	90	540	5.0	0	0	0	2	1	1	3	5.0	6.0	3	2	3.0	3	3	2	2	2
6	5	1	0	210	4.5	0	0	0	3	1	1	3	6.0	7.5	3	1	1.5	4	3	2	2	2
7	4	1	15	615	4.5	0	0	0	3	2	4	3	6.0	7.5	3	1	1.5	4	3	2	2	2
8	1	3	90	540	6.0	0	0	0	1	2	4	3	5.0	6.0	3	1	3.0	4	3	2	2	2
9	1	3	60	600	5.0	0	0	0	3	1	1	3	5.0	7.5	3	2	3.0	4	3	2	2	2
10	1	3	90	270	4.5	0	0	0	3	2	4	3	6.0	7.5	3	1	3.0	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD897672	10087	1	7	4	3	3	4	2	3	2	5	4	1

MACROCHARACTERS

A	B	C	D	E	F	G	H	I	J	K	L
2	4	1	1	2	3	1	1	1	3	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	120	300	6.0	0	0	0	2	1	1	3	6.0	7.5	3	2	3.0	3	3	2	2	2
2	4	1	45	840	4.5	0	0	0	2	1	1	3	4.5	6.0	3	2	3.0	3	3	2	2	2
3	4	1	30	600	4.5	0	0	0	3	1	1	3	6.0	7.5	2	2	4.5	3	3	2	2	2
4	1	3	180	600	6.0	0	0	0	2	1	1	3	6.0	7.5	3	1	3.0	3	3	2	2	2
5	1	3	75	480	6.0	0	0	0	1	1	1	3	5.0	6.0	3	1	3.0	3	3	2	2	2
6	1	3	120	270	5.0	0	0	0	3	1	1	3	6.0	7.5	2	1	4.5	3	3	2	2	2
7	4	1	15	480	6.0	0	0	0	2	1	1	3	6.0	7.5	3	2	1.5	3	3	2	2	2
8	1	3	90	360	6.0	0	0	0	1	1	1	3	4.5	6.0	3	2	1.5	3	3	2	2	2
9	4	1	30	720	4.5	0	0	0	3	1	1	3	6.0	7.5	3	1	3.0	3	3	2	2	2
10	5	1	0	420	4.5	0	0	0	9	9	9	9	0.0	0.0	3	1	0.0	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD897672	10088	1	7	4	3	3	4	2	3	2	5	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	2	3	1	1	1	3	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	75	420	6.0	0	0	0	2	1	1	3	6.0	7.5	3	1	3.0	4	3	2	2	2
2	1	3	90	300	6.0	0	0	0	1	1	1	3	5.0	6.0	3	1	3.0	4	3	2	2	2
3	1	3	60	480	6.0	0	0	0	1	1	1	3	5.0	6.0	3	2	3.0	4	3	2	2	2
4	5	1	0	480	4.5	0	0	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
5	4	1	30	600	4.5	0	0	0	3	1	1	3	6.0	7.5	3	2	3.0	3	3	2	2	2
6	1	3	90	360	6.0	0	0	0	2	1	1	3	6.0	7.5	3	2	1.5	3	3	2	2	2
7	4	1	15	480	6.0	0	0	0	2	1	1	3	6.0	7.5	3	1	1.5	4	3	2	2	2
8	4	1	30	660	4.5	0	0	0	3	1	1	3	5.0	7.5	3	1	3.0	4	3	2	2	2
9	1	3	120	480	6.0	0	0	0	2	1	1	3	6.0	7.5	3	1	3.0	4	3	2	2	2
10	1	3	150	480	6.0	0	0	0	2	1	1	3	6.0	7.5	3	1	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD912656	10089	1	7	4	3	4	4	2	3	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	5	1	2	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	240	540	4.5	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
2	1	3	180	360	5.0	0	2	0	3	1	1	2	10.5	7.5	3	2	1.5	4	3	2	2	2
3	1	3	270	510	5.0	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	4	3	2	2	2
4	1	3	270	570	4.5	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	4	3	2	2	2
5	1	3	90	600	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
6	1	3	240	450	6.0	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
7	9	1	0	90	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
8	1	3	150	420	6.0	0	2	0	3	1	1	2	10.5	9.0	3	2	1.5	4	3	2	2	2
9	1	1	0	180	6.0	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
10	9	1	0	120	6.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD912656	10090	1	7	4	3	4	4	2	3	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	5	1	2	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	240	600	6.0	0	2	0	3	1	1	2	9.0	6.0	3	2	1.5	4	3	2	2	2
2	1	3	270	420	6.0	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	3	3	2	2	2
3	5	3	9	300	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
4	1	3	300	540	6.0	0	2	0	3	1	1	2	9.0	6.0	3	2	1.5	3	3	2	2	2
5	9	1	0	60	6.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
6	1	3	30	480	6.0	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
7	1	3	120	480	4.5	0	2	0	3	1	1	2	6.0	7.5	3	2	1.5	4	3	2	2	2
8	1	3	300	540	6.0	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
9	1	3	210	480	4.5	0	2	0	3	1	1	2	9.0	6.0	3	2	1.5	4	3	2	2	2
10	1	3	240	420	4.5	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD912656	10091	1	7	4	3	4	4	2	3	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	5	1	2	4	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	270	480	6.0	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	4	3	2	2	2
2	1	3	180	360	4.5	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2
3	1	1	300	570	4.5	0	2	0	3	1	1	2	10.5	7.5	3	2	1.5	4	3	2	2	2
4	9	1	0	60	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
5	1	3	240	540	6.0	0	2	0	3	1	1	2	10.5	9.0	3	2	1.5	3	3	2	2	2
6	9	1	0	90	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	2	3	2	2	2
7	1	3	180	420	6.0	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	3	3	2	2	2
8	1	3	330	600	6.0	0	2	0	3	1	1	2	9.0	6.0	3	2	1.5	4	3	2	2	2
9	1	3	210	360	6.0	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	4	3	2	2	2
10	1	1	240	480	6.0	0	2	0	3	1	1	2	9.0	7.5	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD910655	1C092	1	7	4	3	4	5	3	2	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	1	1	1	5	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	270	570	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2
2	1	3	240	480	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2
3	1	3	120	390	4.5	0	2	0	3	1	1	2	7.5	6.0	3	2	3.0	3	3	2	2	2
4	1	3	300	600	4.5	0	2	0	3	1	1	1	6.0	6.0	3	2	3.0	3	3	2	2	2
5	1	3	180	480	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	2	3	2	2	2
6	1	3	210	540	4.5	0	2	0	3	1	1	2	9.0	6.0	3	2	3.0	3	3	2	2	2
7	1	3	300	540	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	3.0	4	3	2	2	2
8	1	3	240	480	4.5	0	2	0	3	1	1	1	9.0	9.0	1	2	3.0	4	3	2	2	2
9	9	1	0	60	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
10	1	3	270	510	6.0	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD910655	1C093	1	7	4	3	4	5	3	2	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	1	1	1	5	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	180	420	4.5	0	2	0	3	1	1	1	6.0	6.0	3	2	1.5	4	3	2	2	2
2	1	3	210	450	4.5	0	2	0	3	1	1	1	7.5	7.5	1	2	3.0	4	3	2	2	2
3	9	1	0	87	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
4	1	3	240	480	4.5	0	2	0	3	1	1	2	7.5	9.0	3	2	3.0	4	3	2	2	2
5	1	3	180	390	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2
6	1	3	300	480	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
7	1	3	210	480	4.5	0	2	0	3	1	1	2	7.5	6.0	3	2	1.5	4	3	2	2	2
8	1	3	270	510	6.0	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
9	1	3	240	600	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2
10	1	3	120	330	4.5	0	2	0	3	1	1	1	6.0	6.0	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
161071	SD910655	10094	1	7	4	3	4	5	3	2	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	1	1	1	5	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	150	390	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2
2	1	3	180	420	4.5	0	2	0	3	1	1	2	9.0	7.5	1	2	3.0	3	3	2	2	2
3	1	3	240	510	4.5	0	2	0	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
4	1	3	150	420	4.5	0	2	0	3	1	1	1	7.5	7.5	3	2	1.5	4	3	2	2	2
5	9	1	0	60	4.5	0	2	0	9	1	1	9	0.0	0.0	3	2	1.5	4	3	2	2	2
6	1	3	180	360	4.5	0	2	0	3	1	1	1	6.0	6.0	3	0	1.5	4	3	2	2	2
7	1	3	270	540	4.5	0	2	0	3	1	1	2	7.5	9.0	3	0	1.5	4	3	2	2	2
8	1	3	210	480	4.5	0	2	0	9	9	9	9	0.0	0.0	3	0	1.5	4	3	2	2	2
9	1	3	300	450	4.5	0	2	0	3	1	1	1	7.5	7.5	3	0	3.0	4	3	2	2	2
10	1	3	240	420	4.5	0	2	0	3	1	1	1	6.0	6.0	3	0	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
21171	NY907284	10099	2	7	0	3	3	3	0	0	0	0	1	0

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	6	0	1	2	1	1	5	0	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	300	600	6.0	0	2	2	3	1	1	1	7.5	7.5	1	0	3.0	4	3	2	2	2
2	1	3	420	900	6.0	0	2	2	3	1	1	1	7.5	7.5	1	0	3.0	3	3	2	2	2
3	1	3	600	990	7.5	0	2	2	3	1	1	1	10.5	10.5	1	0	1.5	4	3	2	2	2
4	1	3	540	1200	6.0	0	2	2	3	1	1	3	9.0	10.5	1	0	3.0	4	3	2	2	2
5	1	3	330	630	3.0	0	2	2	3	1	1	1	9.0	9.0	1	0	3.0	3	3	2	2	2
6	1	3	360	726	6.0	0	2	2	3	1	1	1	7.5	7.5	1	0	3.0	3	3	2	2	2
7	1	3	516	660	4.5	0	2	2	3	1	1	1	9.0	9.0	1	0	3.0	3	3	2	2	2
8	1	3	480	900	4.5	0	2	2	3	1	1	2	7.5	6.0	1	0	1.5	4	3	2	2	2
9	1	3	360	750	6.0	0	2	2	3	1	1	2	10.5	9.0	1	0	3.0	3	3	2	2	2
10	1	3	450	816	4.5	0	2	2	3	1	1	2	9.0	6.0	1	0	3.0	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
230972	NN869643	10100	1	7	3	3	3	5	2	3	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	1	0	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	210	540	9.0	1	2	2	1	1	1	2	6.0	4.5	1	1	3.0	2	1	1	2	2
2	1	3	90	360	9.0	1	1	2	1	1	1	1	6.0	6.0	1	1	4.5	2	1	1	2	2
3	1	3	240	510	6.0	1	2	2	2	1	1	2	6.0	4.5	1	1	3.0	2	2	1	2	2
4	1	3	150	480	6.0	1	2	2	2	1	1	1	6.0	6.0	1	1	3.0	2	2	1	2	2
5	9	1	0	69	6.0	2	2	3	9	9	9	9	0.0	0.0	3	1	1.5	4	3	2	2	2
6	1	3	240	540	7.5	1	2	2	1	1	1	3	6.0	7.5	1	1	4.5	2	1	1	2	2
7	1	3	450	600	6.0	1	2	3	3	1	1	3	7.5	9.0	1	1	3.0	2	1	1	2	2
8	9	1	0	90	6.0	2	2	3	9	9	9	9	0.0	0.0	3	1	1.5	4	3	2	2	2
9	1	3	300	600	6.0	1	2	3	3	1	1	2	6.0	7.5	1	1	3.0	2	2	1	2	2
10	1	3	90	390	6.0	1	2	2	2	1	1	2	9.0	7.5	1	1	3.0	2	1	1	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
230972	NN869644	10101	1	7	3	2	3	3	2	3	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	2	1	2	3	3	1	1	3	3	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	1	0	900	9.0	2	2	2	2	1	1	1	9.0	9.0	3	2	1.5	3	3	2	2	2
2	1	1	0	690	9.0	3	2	2	2	1	1	1	9.0	9.0	3	2	1.5	3	3	2	2	2
3	5	1	0	930	9.0	2	2	3	1	1	1	1	7.5	7.5	3	2	1.5	3	3	2	2	2
4	1	3	120	540	10.5	2	2	2	1	1	1	2	9.0	7.5	3	2	1.5	3	3	2	2	2
5	9	1	0	72	6.0	2	2	3	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2
6	5	1	0	870	9.0	3	2	3	2	1	1	3	9.0	10.5	3	2	1.5	3	3	2	2	2
7	5	1	0	660	10.5	3	2	3	2	1	1	1	10.5	10.5	3	2	1.5	3	3	2	2	2
8	1	3	180	480	9.0	3	2	2	2	1	1	1	9.0	9.0	3	2	1.5	3	3	2	2	2
9	1	3	90	540	10.5	2	2	2	1	1	1	2	9.0	7.5	3	2	1.5	3	3	2	2	2
10	1	1	0	720	9.0	2	2	2	1	1	1	1	7.5	7.5	3	2	1.5	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
230972	NN869645	10102	1	7	2	2	3	2	2	2	2	2	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	2	3	3	1	1	1	3	1

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	1	0	360	9.0	1	2	3	2	1	1	2	9.0	7.5	3	2	1.5	3	3	2	2	2
2	5	1	0	600	9.0	2	2	3	2	1	1	1	9.0	9.0	3	2	1.5	3	3	2	2	2
3	1	3	120	540	7.5	1	2	3	3	1	1	2	9.0	7.5	3	2	1.5	3	3	2	2	2
4	9	1	0	90	6.0	2	2	3	9	9	9	9	0.0	0.0	3	2	1.5	4	5	2	2	2
5	1	3	180	570	10.5	1	2	3	1	1	1	1	9.0	9.0	3	2	1.5	3	3	2	2	2
6	5	1	0	630	9.0	2	2	3	3	1	1	2	10.5	9.0	3	2	1.5	3	3	2	2	2
7	1	1	0	480	9.0	2	2	3	3	1	1	2	10.5	9.0	3	2	1.5	3	3	2	2	2
8	1	1	0	330	10.5	2	2	3	1	1	1	2	9.0	7.5	3	2	1.5	3	3	2	2	2
9	9	1	0	60	6.0	2	2	3	9	9	9	1	0.0	0.0	3	2	1.5	4	3	2	2	2
10	1	3	90	360	9.0	1	2	3	2	1	1	1	9.0	9.0	3	2	1.5	3	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC236250	10104	1	7	3	2	8	4	2	3	2	4	3	2

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	2	1	2	3	3	5	1	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	250	500	12.5	1	2	0	2	1	1	2	12.5	12.5	3	2	4.0	4	2	2	2	2
2	1	3	300	1350	12.5	1	2	0	2	1	1	2	12.5	12.5	3	2	6.0	4	2	2	2	2
3	1	3	500	1250	12.5	1	2	0	1	1	1	2	10.5	10.5	3	2	6.0	4	2	2	2	2
4	1	3	355	1355	12.0	1	2	0	2	1	1	2	12.0	12.0	3	2	4.0	4	2	2	2	2
5	1	3	200	1050	10.0	1	2	0	3	1	1	2	10.5	10.5	3	2	6.0	4	2	2	2	2
6	1	3	200	700	10.5	1	2	0	2	1	1	2	10.5	10.5	3	2	4.0	3	2	2	2	2
7	1	3	250	1150	10.5	1	2	0	2	1	1	1	10.5	10.5	1	2	4.0	3	2	2	2	2
8	1	3	300	1300	12.5	1	2	0	1	1	1	2	10.5	9.0	1	2	6.0	3	2	2	2	2
9	1	3	300	900	12.0	1	2	0	1	1	1	2	10.5	9.0	1	2	6.0	4	2	2	2	2
10	1	3	500	1000	10.0	1	2	0	3	1	1	1	10.5	10.5	1	2	6.0	3	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC237251	10105	1	7	4	3	8	1	5	3	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	4	2	1	1	1	3	1	1	0	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	550	800	5.0	3	2	0	3	1	1	1	7.5	7.5	2	2	3.0	1	1	2	2	2
2	1	3	500	750	9.0	1	1	0	1	1	1	2	6.0	4.5	2	2	3.0	1	1	2	2	2
3	1	3	500	700	9.0	1	1	0	1	1	1	1	6.0	6.0	2	2	4.5	2	1	2	2	2
4	1	3	180	330	6.0	2	2	0	3	1	1	1	7.5	7.5	1	2	3.0	2	2	2	2	2
5	1	3	75	255	6.0	2	2	0	2	1	1	2	6.0	4.5	2	2	4.5	1	1	2	2	2
6	1	3	360	480	7.5	1	2	0	3	1	1	2	9.0	4.5	2	2	3.0	1	1	2	2	2
7	1	3	180	270	6.0	2	2	0	3	1	1	2	9.0	6.0	1	2	3.0	2	2	2	2	2
8	1	3	360	480	9.0	1	2	0	1	1	1	1	7.5	7.5	2	2	6.0	1	1	2	2	2
9	1	3	420	720	9.0	1	1	0	2	1	1	2	9.0	7.5	2	2	3.0	1	1	2	2	2
10	1	3	90	450	7.5	1	2	0	3	1	1	1	6.0	6.0	2	2	3.0	1	1	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC238256	10106	1	7	4	3	8	1	2	2	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	3	1	1	2	0	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	9	1	0	240	10.5	2	2	1	9	9	9	9	0.0	0.0	3	2	3.0	4	3	2	2	2
2	1	1	0	90	10.5	1	2	1	9	9	9	9	0.0	0.0	3	2	3.0	3	2	2	2	2
3	3	1	0	450	12.0	1	2	1	2	1	1	2	12.0	12.0	3	2	3.0	3	2	2	2	2
4	1	3	120	420	12.0	1	2	2	2	1	1	1	12.0	12.0	3	2	4.5	3	2	2	2	2
5	1	3	150	780	10.5	2	2	2	2	1	1	2	10.5	9.0	2	2	3.0	3	2	2	2	2
6	3	3	0	750	12.0	1	2	1	1	1	1	2	10.5	9.0	2	2	3.0	3	2	2	2	2
7	3	3	0	390	12.0	1	2	2	2	1	1	2	12.0	10.5	3	2	3.0	4	3	2	2	2
8	1	3	120	540	10.5	1	2	2	2	1	1	1	10.5	10.5	2	2	4.5	3	2	2	2	2
9	3	3	60	360	9.0	2	2	2	3	1	1	2	10.5	9.0	3	2	3.0	4	3	2	2	2
10	1	3	150	570	12.0	1	2	2	2	1	1	1	12.0	12.0	2	2	4.5	3	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC246237	10112	1	7	3	2	6	5	2	2	2	2	1	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	2	1	1	2	1	5	2	2	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	9	1	0	120	6.0	2	2	1	9	9	9	9	0.0	0.0	3	9	1.5	4	3	2	2	2
2	5	1	0	600	9.0	2	2	3	9	9	9	9	0.0	0.0	3	2	3.0	4	3	2	2	2
3	1	3	120	400	7.5	1	2	2	3	1	1	2	9.0	7.5	1	2	3.0	3	2	2	2	2
4	1	3	120	300	7.0	1	2	2	3	1	1	1	9.0	9.0	1	2	3.0	2	2	2	2	2
5	1	3	300	600	9.0	1	2	2	2	1	1	1	9.0	9.0	1	2	3.0	3	2	2	2	2
6	1	3	200	700	12.5	1	2	2	2	1	1	2	12.0	10.5	1	2	3.0	3	2	2	2	2
7	1	3	200	500	12.5	1	2	2	2	1	1	1	12.0	12.0	1	2	3.0	3	2	2	2	2
8	1	3	120	330	12.0	1	2	2	1	1	1	1	10.5	10.5	1	2	3.0	3	2	2	2	2
9	1	3	500	1000	15.0	1	2	2	1	1	1	3	12.5	15.0	1	2	3.0	3	2	2	2	2
10	5	1	0	750	10.5	2	2	3	2	1	1	2	10.5	10.5	3	2	3.0	3	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC246237	10113	1	7	3	2	6	4	2	2	2	4	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	1	2	1	1	0	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	400	650	5.0	1	2	9	3	1	1	2	6.0	4.5	1	2	6.0	2	2	2	2	2
2	1	3	650	400	6.0	1	2	9	2	1	1	1	6.0	6.0	1	2	6.0	2	2	2	2	2
3	1	3	90	300	6.0	1	2	9	2	1	1	2	6.0	4.5	1	2	4.5	2	2	2	2	2
4	1	3	240	390	3.0	1	2	9	3	1	1	2	4.5	3.0	3	2	3.0	3	2	2	2	2
5	1	3	390	640	6.0	1	2	9	2	1	1	2	6.0	3.0	1	2	6.0	2	2	2	2	2
6	1	3	900	1200	6.0	1	2	9	2	1	1	2	6.0	3.0	3	2	4.5	2	2	2	2	2
7	1	3	720	970	6.0	1	2	9	2	1	1	1	6.0	6.0	3	2	4.5	2	2	2	2	2
8	1	3	600	900	6.0	1	2	9	1	1	1	1	4.5	4.5	1	2	6.0	2	2	2	2	2
9	1	3	240	480	6.0	1	2	9	2	1	1	2	6.0	3.0	1	2	6.0	2	2	2	2	2
10	1	3	30	300	6.0	1	2	9	1	1	1	2	6.0	4.5	1	2	6.0	2	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC247235	10115	2	7	3	2	3	1	2	3	2	1	1	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	1	3	1	5	0	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	150	600	6.0	1	2	2	2	1	1	1	6.0	6.0	3	2	3.0	3	2	2	2	2
2	1	3	450	810	4.5	1	2	1	3	1	1	2	6.0	4.5	1	2	4.5	2	1	2	2	2
3	1	3	900	1140	6.0	1	2	2	2	1	1	2	6.0	4.5	1	2	4.5	2	1	2	2	2
4	1	3	180	510	4.5	1	2	2	2	1	1	2	4.5	3.0	1	2	4.5	2	2	2	2	2
5	1	3	350	525	6.0	1	2	2	2	1	1	1	6.0	6.0	1	2	3.0	1	1	2	2	2
6	1	3	175	300	6.0	1	2	1	2	1	1	1	6.0	6.0	1	2	3.0	1	1	2	2	2
7	1	3	200	550	10.5	1	1	2	1	1	1	2	6.0	4.5	1	2	4.5	2	2	2	2	2
8	1	3	300	500	6.0	1	2	1	3	1	1	1	4.5	4.5	1	2	4.5	2	2	2	2	2
9	1	3	450	850	6.0	1	2	2	2	1	1	2	6.0	4.5	1	2	3.0	3	2	2	2	2
10	1	3	100	500	10.5	1	1	2	1	1	1	1	6.0	4.5	1	2	3.0	1	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC247235	10116	2	7	3	2	6	1	2	1	2	3	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	5	1	1	2	3	1	5	3	1	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	300	750	12.0	1	2	2	3	1	1	2	15.0	12.0	3	2	3.0	4	2	2	2	2
2	1	3	330	600	12.0	1	2	2	2	2	2	3	12.0	15.0	1	2	3.0	4	3	2	2	2
3	1	3	510	810	10.5	1	2	2	2	2	1	3	10.5	12.0	1	2	4.5	3	2	2	2	2
4	5	1	0	720	10.5	2	2	3	2	1	1	1	10.5	10.5	3	2	3.0	3	3	2	2	2
5	1	3	480	1080	12.0	1	2	2	2	1	1	2	12.0	10.5	1	2	3.0	4	3	2	2	2
6	1	3	240	540	9.0	1	2	2	3	2	1	2	10.5	9.0	3	2	3.0	3	2	2	2	2
7	1	3	420	780	10.5	1	2	3	3	1	1	3	12.0	15.0	3	2	3.0	3	3	2	2	2
8	1	3	300	900	12.0	1	2	2	2	2	1	2	12.0	12.0	3	2	4.5	4	3	2	2	2
9	1	3	270	580	9.0	2	2	2	3	1	1	3	10.5	15.0	1	2	3.0	4	3	2	2	2
10	9	1	0	240	6.0	2	2	3	9	9	9	9	0.0	0.0	3	2	1.5	4	3	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC263214	10120	2	7	3	2	6	1	2	4	2	3	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	5	2	9	1	1	3	1	1	0	0	5	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	1	0	240	4.5	1	2	9	3	1	1	1	6.0	6.0	2	2	7.5	2	2	2	2	2
2	1	3	150	330	4.5	1	2	9	3	1	1	1	6.0	6.0	2	2	7.5	2	2	2	2	2
3	1	1	0	420	7.5	1	2	9	3	1	1	2	7.5	6.0	2	2	7.5	2	2	2	2	2
4	1	3	240	540	7.5	1	2	9	2	1	1	1	7.5	7.5	2	2	3.0	2	2	2	2	2
5	1	3	120	270	6.0	1	2	9	2	1	1	1	6.0	6.0	3	2	3.0	2	2	2	2	2
6	1	3	150	450	6.0	1	2	9	2	1	1	1	6.0	6.0	3	2	4.5	2	2	2	2	2
7	1	1	0	300	7.5	1	2	9	2	1	1	2	7.5	4.5	2	2	7.5	2	2	2	2	2
8	1	3	120	360	6.0	1	2	9	3	1	1	2	7.5	6.0	3	2	6.0	2	2	2	2	2
9	1	3	90	300	6.0	1	2	9	2	1	1	1	6.0	6.0	3	2	3.0	2	2	2	2	2
10	1	3	120	450	7.5	1	2	9	2	1	1	1	7.5	7.5	2	2	4.5	2	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
240972	NC255217	10125	1	7	3	3	3	4	2	2	2	4	4	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	3	1	1	2	3	1	0	1	1	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	330	720	7.5	2	2	9	3	1	1	3	9.0	10.5	1	2	6.0	3	2	2	2	2
2	1	3	150	450	10.5	1	2	9	1	2	1	1	9.0	9.0	1	2	6.0	3	2	2	2	2
3	1	3	300	600	9.0	1	2	9	2	1	1	3	9.0	10.5	1	2	4.5	3	2	2	2	2
4	5	1	0	420	9.0	2	2	9	2	1	1	1	9.0	9.0	3	2	3.0	4	2	2	2	2
5	1	3	210	630	7.5	2	2	9	3	1	1	2	9.0	7.5	1	2	4.5	3	2	2	2	2
6	1	3	120	450	7.5	1	2	9	2	1	1	1	7.5	7.5	1	2	4.5	4	2	2	2	2
7	1	3	390	810	10.5	1	2	9	2	2	1	1	10.5	10.5	1	2	3.0	4	2	2	2	2
8	9	1	0	210	6.0	3	2	9	9	9	9	9	0.0	0.0	3	2	1.5	4	2	2	2	2
9	1	3	270	510	9.0	2	2	9	3	1	1	1	10.5	9.0	1	2	6.0	3	2	2	2	2
10	1	3	210	480	9.0	2	2	9	2	1	1	2	9.0	9.0	1	2	4.5	3	2	2	2	2

DATE	GRIDREF	NUM	HC	A	B	C	D	E	F	G	H	I	J	K
190673	NY847122	10150	1	7	3	3	0	1	2	2	2	3	3	1

MACROCHARACTERS	A	B	C	D	E	F	G	H	I	J	K	L
	2	4	1	1	2	1	3	5	0	2	4	0

TRICHOMES

TN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1	3	180	330	6.0	3	2	0	2	1	1	1	6.0	6.0	1	2	3.0	2	1	1	2	2
2	1	3	120	330	7.5	3	2	0	2	1	1	1	7.5	7.5	1	2	1.5	2	1	1	2	2
3	1	3	72	600	7.5	3	2	0	3	1	1	1	9.0	9.0	1	2	2.5	2	1	1	2	2
4	1	3	150	540	7.5	3	2	0	2	1	1	1	7.5	7.5	1	2	3.0	2	1	1	2	2
5	1	3	450	576	6.0	3	2	0	2	1	1	1	6.0	6.0	1	2	3.0	2	1	1	2	2
6	1	3	240	510	3.0	3	2	0	3	1	1	1	6.0	6.0	1	2	2.5	2	1	1	2	2
7	5	3	0	900	6.0	3	2	0	2	1	1	1	6.0	6.0	1	2	3.0	2	1	1	2	2
8	1	3	150	195	9.0	3	2	0	2	1	1	1	9.0	9.0	1	2	3.0	2	1	1	2	2
9	1	3	300	600	9.0	3	2	0	2	1	1	1	9.0	9.0	1	2	3.0	2	1	1	2	2
10	1	3	120	510	9.0	3	2	0	2	1	1	1	9.0	9.0	1	2	3.0	2	1	1	2	2

PLEUROCLADIA LACUSTRIS A. BRAUN (PHAEOPHYTA) — A NEW BRITISH RECORD.

S. M. KIRKBY[1], D. J. HIBBERD[2] and B. A. WHITTON[1]

1. Department of Botany, University of Durham, Durham City.
2. Culture Centre of Algae and Protozoa, 36, Storey's Way, Cambridge, CB3 ODT.

The great majority of species of brown algae are found either in marine or brackish habitats. Nevertheless, there are a few freshwater forms, and in view of their apparent rarity, these have always been of considerable interest to algologists. The present note describes what we believe to be the first record of *Pleurocladia lacustris* A. Braun in Britain.

Bourrelly's (1968) flora lists four genera of brown algae represented in freshwaters: *Bodanella, Pleurocladia, Heribaudiella, Lithoderma.* Among these the forms included by various authors under either *Heribaudiella* or *Lithoderma* are certainly very similar, if not identical (see Hamel, 1931-9). *Bodanella* is monospecific (Bourrelly, 1968), while the various forms of *Pleurocladia* have also sometimes been regarded as a single species (Wilce, 1966). The known species of freshwater brown algae therefore fall into three distinct groups, including very few (possibly only three) species. In Britain, *Lithoderma fluviatile* Aresch. (= *Heribaudiella* (Aresch.) Svedelius) has been recorded from several rivers in North Devon (Fritsch, 1929), but there are apparently no records for either *Bodanella* or, prior to this, for *Pleurocladia.*

HABITAT

Description of site

The alga was found (by S. M. K.) in the larger of the two main ponds at Brasside, County Durham (National Grid reference NZ 292454, altitude 60m).

The ponds at Brasside have arisen as the result of extensive excavations into the laminated clays of the old submerged valley of the River Wear (Maling, 1955). There has probably been some open water in the area for fifty years, with fish stocking having been carried out for at least half that time. Part of the area near the large pond was used in the 1950's by British Rail and the War Department as a dump, while until recently many old bottles and tins were evident on the floor of the small pond. The ponds have reached their present general form only within the last twenty years, the final major change taking place in 1966 when the smaller pond became connected with the larger one, thus slightly raising the level of the water in thee latter. There is a small outlet stream from the large pond running into the River Wear.

Water Chemistry

Analysis of water collected from the vicinity of the *Pleurocladia* on 11 September 1972, and treated by the methods given in Holmes *et al.* (1972), gave the following results:

Na	28.3 mg/l	Al	<0.03 mg/l
K	13.5 mg/l	Pb	0.002 mg/l
Mg	82.0 mg/l	Cl	41.0 mg/l
Ca	51.7 mg/l	Si	0.90 mg/l
Zn	0.002 mg/l	pH	7.8
Cu	0.05 mg/l	conductivity:	784 micro-mhos
Mn	0.13 mg/l	optical density at 420 nm:	0.021
Fe	0.17 mg/l		

The mud at the edge and bottom of the site with *Pleurocladia* consisted of easily disturbed black silt. Such disturbance caused a moderate smell of H_2S.

Associated species

Thalli of *Pleurocladia* were found to be frequently attached to rotting leaves of *Typha latifolia* L. at one site during September and early October 1972. Other algal epiphytes with conspicuous thalli on such leaves were: *Chaetophora pisiformis* (Roth) C. A. Ag. (abundant), *Coleochaete scutata* de Bréb. (occasional), *Gloeotrichia pisum* (C. A. Ag.) Thuret (rare). Leaves which were dead, but not yet rotting, did not show any obvious *Pleurocladia* thalli, although colonies of *Chaetophora* were usually evident. Inspection of rotting leaves of *Typha* from other parts of this and the adjacent pond did not reveal any other sites with *Pleurocladia*. This particular site is also the only one for which *Gloeotrichia* has so far been recorded from this pond.

Typha latifolia grows at this site in shallow water overlying the black mud, together with *Myriophyllum spicatum* L. and *Potamogeton pectinatus* L. and with *Entermorpha flexuosa* (Wulfen *ex* Rdh.) J. Ag. frequent in summer and autumn at the surface of the pond. Colonies of *Gloeotrichia pisum* and *Chaetophora pisiformis* have been recorded here in late summer as epiphytes on the fine-leaved flowering plants since 1965. However leaves of *Typha* have not been inspected previously, so it is not known whether *Pleurocladia* was also present.

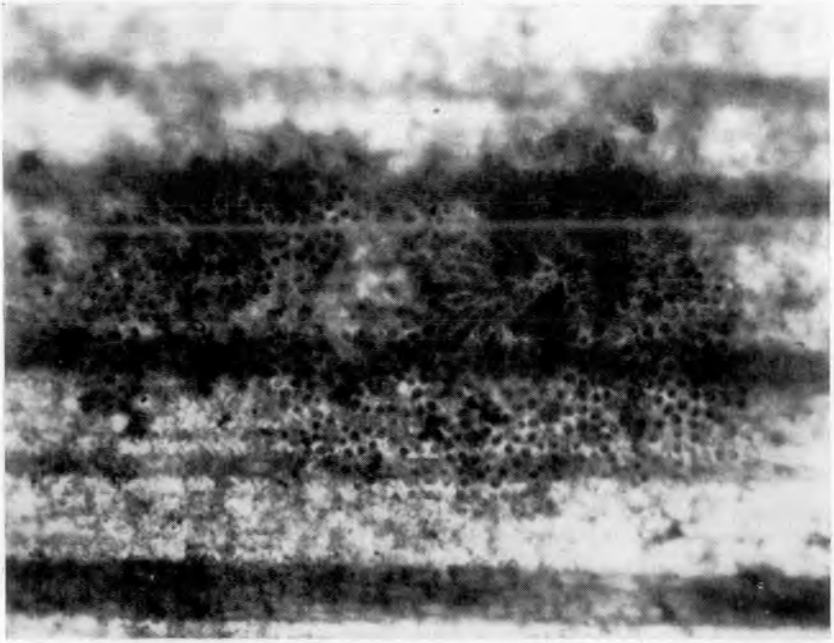


Fig. 1. Two thalli of *Pleurocladia* on a rotting *Typha* leaf. Magnification x 140.

DESCRIPTION OF ALGA

The material from Brasside pond corresponded well with descriptions in the literature such as those given by Pascher (1925), Waern (1952) and Wilce (1966). The excellent series of diagrams shown by Wilce would in general be equally applicable to the Brasside material. However, unlike the marine populations of

Wilce or the brackish one of Waern, but like the majority of previous freshwater records, the Brasside material showed marked calcification in old thalli. Associated with this calcification, old thalli appeared a pale golden brown to the naked eye, in contrast to the rather deeper brown shown by young thalli.

The alga formed thalli up to 3 mm diameter on the rotting leaves (Fig. 1) though they were usually somewhat smaller. The thickness of old thalli, excluding any hairs, was 200-300 μm. The hairs however, which were most evident on thalli of intermediate age, often exceeded twice this length. Although thalli were scattered over most of the surfaces of rotting leaves, some tendency to aggregation was usually apparent, suggesting that secondary colonization may play a role in its spread on the leaf.

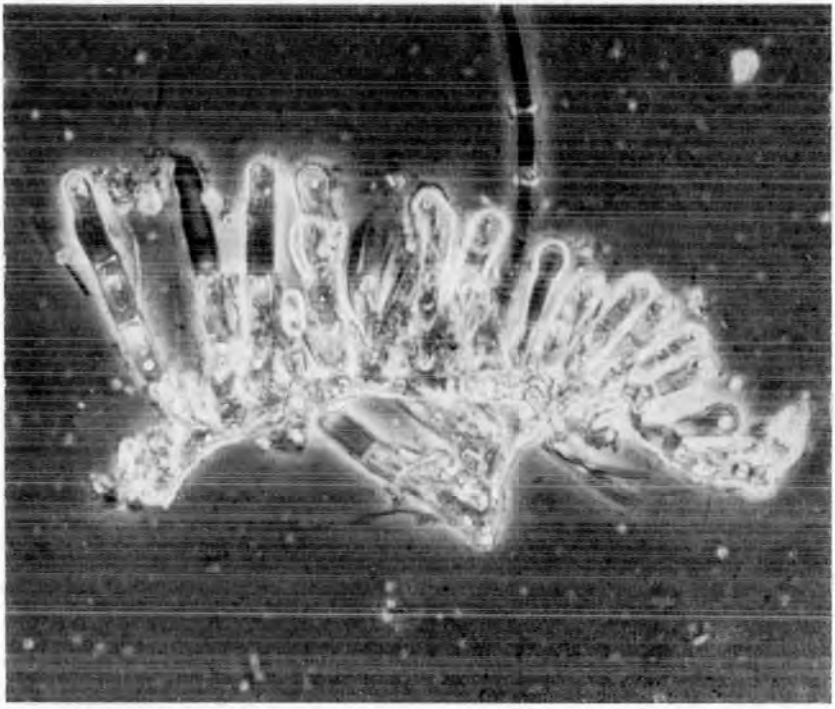
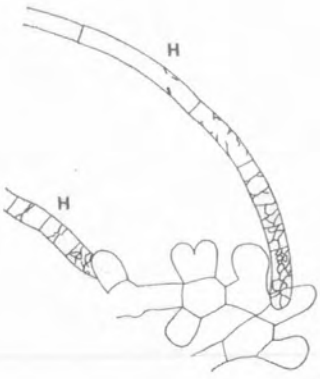


Fig. 2. Part of a young thallus, including base of a hair. Magnification x 400

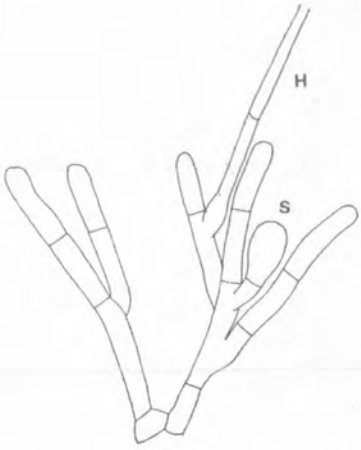
The filaments of Brasside material were mostly 10-16 μm wide, while ripe sporangia were 18-24 μm and 28-38 μm long (Fig. 2, 3). The dimensions of both filaments and sporangia thus lie in about the middle of the ranges given by other authors and summarized by Wilce (1966). Filaments of old calcified thalli were markedly wider than those of young ones (means of 15 and 11 μm, respectively). One slight difference from the material described by Wilce is that the Brasside alga never showed such marked indentation at the cross-walls of the filaments. The hairs (Fig. 3C) in particular, never showed the articulated appearance illustrated by Wilce.

A

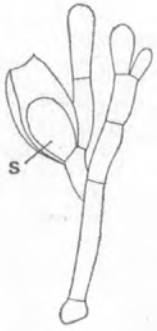
B



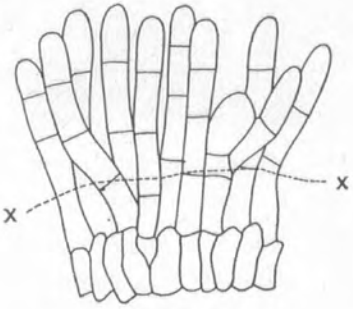
C



D



E



F

0 50 µm

FIG. 3

Fig. 3 *Pleurocladia lacustris*

A. Part of a very young thallus.
B. Appearance of the pseudoparenchymatous basal filaments in vertical view.
C. Part of a relatively young thallus showing the development of vacuoles in the cells of the basal regions of long hairs (H).
D. Detached vertical filaments showing the mode of branching, a sporangium (S) and the proximal part of a hair (H).
E. Branched vertical filament bearing a sporangium sheathed by a "husk," the wall of an old discharged sporangium.
F. Part of a very old thallus. The outer limit of calcification (X-X) approximately corresponds with distal ends of the second tier of cells; the basal tier is much more heavily calcified than the second one, and the cell outlines are therefore distinguishable only with difficulty.

The pattern of calcification shown by the *Pleurocladia* thalli from Brasside was striking. Young thalli quite lacked any calcification, while in old ones the parts of the filaments furthest from the leaf surface were also quite free (Fig.2). The calcification in the lower parts of the thallus occurred in distinct layers parallel to the surface. Old thalli typically showed two such layers, but occasionally three were present. Each layer corresponded approximately (but not exactly) with a layer of cells. The innermost layer was much more heavily calcified than the outer one(s).

DISCUSSION

The known world wide distribution of *Pleurocladia lacustris* has been summarized by Wilce (1966). There are about two dozen sites for which it is known, and about half of these are freshwater. However, as Wilce points out, the plants are small and easily overlooked, so it is uncertain just how rare it really is. Of the freshwater forms, it would seem likely to prove the most useful for culture and general research purposes, so it is hoped that botanists will be on the lookout for this plant at other likely sites. Several features of its occurrence at Brasside resemble features described for other sites, so we can start to build up a picture of the type of site where it would seem particularly promising to look for this plant.

Like the Brasside pond, the Baltic sites described by Waern (1962) are associated with shallow eutrophic lakes in clay districts. On the other hand, there seems to be no marked tendency for the plant to grow as an epiphyte on particular species, as a range of host plants have been quoted. Most, however, are monocotyledons with wide leaves, *Phragmites* being quoted most frequently. Most, if not all, the freshwater records are from calcareous waters. There are obvious floristic similarities between the other plants present at the Brasside pond and those reported from the other sites. One minor one is the association with Rivulariaceae at Brasside (*Gloeotrichia pisum*) and at least four other sites in the literature. J. B. Petersen in Wesenberg-Lund (1917) described a freshwater population from Lake Furesø on stones together with *Rivularia*. Both Israelsson (1938, in Lake Erken, Sweden) and Pankow *et al.* (1971, in Saaler Bodden, D.D.R.) report *Pleurocladia*, *Rivularia* and *Gloeotrichia pisum* as all occurring on *Phragmites*, while Wilce mentioned that in the marine population growing on a limestone shore the belt of *Pleurocladia* sometimes merged with the *Calothrix* belt.

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We are most grateful to Mr. G. Whitfield, the owner of the pond, for permission to visit the site, to Dr. J. C. Coulson, Mr. M. J. Hudson and Mr. J. Richardson for historical information concerning Brasside ponds, and to Mrs. J. A. Moore for confirming that no material of this alga (freshwater or marine) from Britain has been previously deposited at the British Museum (Natural History).

AVAILABILITY OF MATERIAL

It is suggested that anyone wishing to visit the large pond at Brasside for research purposes should first contact one of the authors at the Department of Botany, Durham University. Material from this site will be deposited in the Herbarium of the British Museum (Natural History).

PLANTS OF THE RIVER TYNE AND FUTURE WATER TRANSFER SCHEME

N. T. H. HOLMES, E. J. H. LLOYD, M. POTTS and B. A. WHITTON

Department of Botany, University of Durham, Durham City

Few rivers in Britain have been studied in sufficient detail for the effects on their vegetation of any future changes in management either to be forecast or monitored as they occur. Nevertheless inspection by the authors of various rivers in South-East Scotland and North-East England has shown that there are sometimes considerable floristic differences when passing from one river to the next. So any future changes in management, such as the mixing of different river waters, may well be expected to bring about floristic changes in the rivers concerned.

It is at present far from clear to what extent these floristic differences occurring between rivers in the region, or on passing down individual ones, are associated with differences in physical, chemical or historical factors. The changes in macrophyte flora on passing down the Wear (Whitton & Buckmaster, 1970) are certainly in part due to decreased flow rates and associated changes in the substratum. Nevertheless the entry of the Skerne into the Tees, which causes a marked chemical, but little physical, change in the latter river, brings about a