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MORPHOLOGICAL VARIATION IN THE GENUS ACROCHAETIUM (RHODOPHYTA, NEMALIALES)

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SUMMARY

An evaluation is given of some morphotaxonomic criteria used for specific distinction in the genus *Acrochaetium*. 59 isolates from the European Atlantic coast were compared after having been cultured under uniform conditions. As a result, three species are distinguished, primarily on the characters cell length, arrangement and length of the monosporangia. Cell diameter and shape of the monosporangia and monosporangial stalk cell may sometimes be used as additional characters for identification purposes.

Recognized taxa have not undergone formal nomenclatural revision, and their identification has been provisional; they are: *A. zosterae* Papenfuss, *A. nemalionis* (De Notaris) Bornet, and *A. daviesii* (Dillwyn) Nägeli. Because of large intraspecific variation, differences between the three are not very clear cut. Sexual life histories are known from culture in the first two species. Comparison with relevant literature shows some of the commonly used criteria for species distinction to be of little value.

The position of the genus *Acrochaetium* within the family Acrochaetiaceae is briefly discussed.

1. INTRODUCTION

The genus *Acrochaetium* Nägeli comprises species of acrochaetioid morphology with parietal chromatophores provided with pyrenoids, and practically isomorphic generations. It was defined in this sense by STEGENGA & VROMAN (1977); in terms of described species it forms one of the largest genera in the family Acrochaetiaceae. The other major genus is *Chromastrum* Papenfuss, which has been surveyed in more detail elsewhere (STEGENGA & MULDER 1979).

Life history studies in the genus *Acrochaetium* have been performed on *A. dasyae* Collins (STEGENGA & BORSJE 1976), and on *A. asparagopsis* (Chemin) Papenfuss (MAGNE 1977 – as *A. asparagopsidis*); the latter species is almost completely endophytic, but its life history pattern and its ability to form erect filaments in culture appear to warrant its inclusion in *Acrochaetium*. Excepting these two species and a few clones of *A. nemalionis* (see below) we have had disappointingly little success in life history work on this genus; several isolates from the European Atlantic coast could not be induced to form any reproductive structures other than monosporangia, such in marked contrast to *Chromastrum* species (STEGENGA & MULDER 1979). On the other hand, several species of *Acrochaetium* have been described from the field with gametangia, carposporophytes and tetrasporangia, thus suggesting that they may complete their sexual cycles in nature (e.g. WOELKERLING 1971).

The lack of details on life histories forced us to study systematic relationships

within the genus *Acrochaetium* by means of comparative morphology of a large number of isolates. It should be noted here, that these clones have been selected as *Acrochaetium* species on purely vegetative characters, i.e. parietal chromatophores with a pyrenoid, and a multicellular filamentous base formed after non-septate germination. STEGENGA & VAN WISSEN (1979) have pointed out that this characteristic is not sufficient to separate *Acrochaetium* from the tetrasporophytic part of *Kylinia*; it may be taken as an indication of the narrow relationships between *Acrochaetium* and *Kylinia*. Some isolates, originally included in the present study, have later been left out because of proved conspecificity with *Kylinia rosulata*; in this case the abundance of unicellular hairs was also in marked contrast with the other species under investigation.

Material in the present study originated from France (Brittany), the Netherlands, and the Swedish west coast. On examination of the relevant literature (ROSENVINGE 1909, HAMEL 1928a, KYLIN 1944, DIXON & IRVINE 1977), it is concluded that some 8–12 species of *Acrochaetium* occur in this region, not counting completely endophytic and endozoic species. The species are distinguished on various characteristics, i.e. cell dimensions, monosporangial dimensions and arrangement, branching, and characters of the prostrate part (epiphytic or endophytic). With the exception of the prostrate part, we have compared these characters for some or all of 59 isolates.

2. MATERIALS AND METHODS

59 clones were collected in the course of several years since 1965, and kept in store at 12 °C, 12/12 daylength regime. Isolates originated from Sweden (County of Bohuslän), the Netherlands (Province of Zeeland) and France (Department of Finisterre); localities are listed, together with data on morphology and substrate, in *table 1*.

All strains were compared after having been grown under uniform conditions (16 °C, 12/12 daylength) during 2–3 weeks, or longer if necessary for monosporangium formation; measurements were made on plants that had grown from monospores in that time.

From these strains 6 were selected and studied for growth characteristics and morphological variability under two temperatures (8 and 16 °C) and two daylengths (8/16 and 16/8 photoperiods).

Strains 328 (gametophyte) and 347 (corresponding tetrasporophyte) were tested for their reproductive capacity in short and long day combined with temperatures 4, 8, 12 and 16 C. Light intensity in all experiments was approximately 1750 lx; the culture medium, an enriched seawater according to PROVASOLI (1968) was renewed every two weeks. Plants were grown in plastic petri-dishes, with glass cover slips to serve as a substrate for the plants.

Identification of recognized taxa has taken place using literature concerning the North Atlantic Ocean.

Table 1. Morphological characteristics of 59 isolates grown at 16 C, 12/12 daylength. All measures in µm, averages of 5 plants. Monosporangial arrangement: s. = single, c. = clustered, c.c. = clustered, monosporangia with cup-shaped pedicel. Origin: F. = France, N. = Netherlands, S. = Sweden. Nomenclature of substrates according to PARKE & DIXON 1976.

strain no.	cell length	cell diameter	monosporangial length	monosporangial diameter	monosporangial arrangement	substrate	origin
160	53.4	9.6	21.6	14.4	s.	Dasya baillouviana	N., Kanaal door Zuid-Beveland
161	46.8	9.7	24.5	13.4	s.	Dasya baillouviana	N., Kanaal door Zuid-Beveland
162	37.0	6.8	26.3	12.1	s.	Dasya baillouviana	N., Kanaal door Zuid-Beveland
163	48.5	8.3	27.5	14.6	s.	Dasya baillouviana	N., Kanaal door Zuid-Beveland
164	63.3	10.0	24.7	14.5	s.	Dasya baillouviana	N., Kanaal door Zuid-Beveland
165	35.0	7.6	26.0	12.6	s.	Dasya baillouviana	N., Gat van Ouwerkerk
166	41.9	9.0	21.7	12.8	s.	Dasya baillouviana	N., Gat van Ouwerkerk
167	44.4	9.0	26.4	13.6	s.	Dasya baillouviana	N., Gat van Ouwerkerk
168	46.0	9.3	25.3	13.6	s.	Dasya baillouviana	N., Gat van Ouwerkerk
169	45.1	7.8	25.5	11.6	s.	Dasya baillouviana	N., Gat van Ouwerkerk
170	53.3	9.5	26.8	12.1	s.	culture obtained	tetrasporophyte of 163 × 167
171	52.0	10.2	23.4	11.3	s.	culture obtained	tetrasporophyte of 161 × 165
172	57.7	10.6	28.2	13.0	s.	culture obtained	tetrasporophyte of 162 × 166
203	48.0	9.2	23.0	11.5	s.	Chondria dasyphylla	N., Yerseke
211	54.7	9.8	29.8	12.5	s.	Chondria dasyphylla	N., Yerseke
212	50.0	8.5	26.0	12.0	s.	Chondria dasyphylla	N., Yerseke
221	59.1	8.9	17.9	10.5	c.	Codium fragile	N., Sas van Goes
222	39.5	8.3	18.8	12.1	c.	Codium fragile	N., Sas van Goes
224	41.7	9.3	18.9	10.9	c.	Laminaria saccharina	N., Sas van Goes
256	32.3	7.8	13.5	9.0	c.c.	Polysiphonia nigrescens	N., Sas van Goes
267	23.8	9.3	12.0	8.8	c.c.	Rhodomela sp.	F., Roscoff
269	20.8	9.7	11.8	9.8	c.	Lomentaria articulata	F., Roscoff
270	28.3	9.0	11.8	8.6	c.c.	Nitophyllum punctatum	F., Roscoff
280	32.0	11.0	17.8	8.7	c.	Nemalion helminthoides	F., Ile de Batz
281	27.3	10.3	18.4	11.5	c.	Nemalion helminthoides	F., Ile de Batz
282	32.1	11.3	20.4	10.4	c.	Nemalion helminthoides	F., Ile de Batz
283	31.5	8.6	11.5	9.1	c.c.	Cladophora rupestris	F., Ile de Batz
284	20.4	8.5	13.9	9.3	c.	Cladophora rupestris	F., Ile de Batz
285	19.5	8.5	13.7	10.4	c.c.	Cladophora rupestris	F., Ile de Batz
289	31.9	11.6	18.5	10.7	c.	Nemalion helminthoides	F., Ile de Batz
293	24.5	10.0	12.6	8.6	c.c.	Heterosiphonia plumosa	F., Trémazan
294	28.1	12.3	19.0	11.7	c.	Cladostephus spongiosus	F., Trémazan
298	35.4	9.4	11.5	9.6	c.c	Corallina sp.	F., Trémazan
304	38.1	11.6	21.4	11.0	c.	Nemalion helminthoides	F., Trémazan
305	42.6	11.0	19.5	11.0	c.	Nemalion helminthoides	F., Trémazan
307	32.1	10.9	19.2	12.9	c.	Gastroclonium ovatum	F., Trémazan
308	21.4	7.8	10.9	9.5	c.c.	Lomentaria articulata	F., Roscoff
309	23.0	8.8	12.9	10.0	c.c.	Lomentaria articulata	F., Roscoff

Table 1. Continued.

strain no.	cell length	cell diameter	monosporangial length	monosporangial diameter	monosporangial arrangement	substrate	origin
311	21.7	11.5	15.3	12.3	c.	Nemalion helminthoides	F., Trémazan
312	31.4	11.8	20.1	11.2	c.	Nemalion helminthoides	F., Trémazan
315	39.5	11.5	21.7	11.8	c.	Codium fragile	F., Trémazan
316	27.4	9.8	17.6	12.2	c.	Codium fragile	F., Trémazan
326	35.3	9.0	12.3	9.2	c.c.	Ceramium sp.	F.,Trémazan
328	25.9	13.0	18.3	12.5	c.	Ceramium sp.	F., Trémazan
330	25.8	8.8	12.5	8.6	c.	Ceramium echionotum	F., Trémazan
345	51.0	8.5	30.5	11.7	s.	Polysiphonia nigrescens	N., Bruinisse (Grevelingen)
347	24.2	10.8	16.9	12.7	c.	culture obtained	tetrasporophyte of 328
360	25.1	7.8	13.0	8.3	c.c.	Delesseria sanguinea	S., Kristineberg
362	20.4	7.2	12.2	9.3	c.c	Lomentaria orcadensis	S., Kristineberg
383	30.4	9.8	13.2	8.0	c.c.	Ahnfeltia plicata	S., Kristineberg
385	44.5	7.4	24.0	11.4	s.	Mesogloia vermiculata	S., Kristineberg
386	48.8	7.8	25.6	10.6	c.	Mesogloia vermiculata	S., Kristineberg
387	34.2	9.0	13.4	7.7	c.	Cladophora sp.	S., Kristineberg
394	37.5	8.5	23.8	11.1	c.	Mesogloia vermiculata	S., Kristineberg
395	43.4	7.8	25.9	10.8	c.	Mesogloia vermiculata	S., Kristineberg
418	52.8	7.5	25.5	11.3	s.	Mesogloia vermiculata	S., Kristineberg
419	40.6	6.7	22.8	10.2	s.	Mesogloia vermiculata	S., Kristineberg
506	24.1	6.9	12.7	7.2	c.	Chaetomorpha sp.	F., Ile de Batz
507	27.5	7.5	13.4	8.6	c.	Chaetomorpha sp.	F., Ile de Batz

3. RESULTS

3.1. Interclonal variation and species delimitation

Results of measurements on 59 clones are given in *table 1*, together with data on origin and substrate of the isolates. Cell length, cell diameter and monosporangial dimensions are averages of measurements on five plants. Arrangement of monosporangia has arbitrarily been divided into two classes: clustered or non-clustered. Plants bearing their sporangia usually singly or in pairs, or occasionally with three together, are considered to have a non-clustered arrangement; when monosporangia are usually borne with three or more on small determinate branchlets, this is called a clustered arrangement; the decision has been made upon direct observation and afterwards checked by comparison of photographs of the isolates. Size of the clusters, which may vary considerably, is not included in this table.

Still another character has been used in connection with the monosporangia,

namely the shape of the subsporangial stalk cell. This cell is typically very short and cup-shaped in some clones, gradually widening from its base to the side adjacent to the sporangium (*fig. 13*); in the other clones this pedicel is longer and more cylindrical in shape. The cup-shaped pedicels as a rule bear only one sporangium, whereas cylindrical stalk cells often bear two sporangia when in terminal position. In connection with the cup-shaped stalk cell, the monosporangium is somewhat dome-shaped, whereas in other cases it is ovoid or elongate ellipsoid; the monosporangium together with a cup-shaped stalk cell may give the impression of a bisporangium.

Concerning the morphological data, there are significant positive correlations (Spearman's rank-correlation test, $\alpha = 0.05$) between monosporangial length and diameter ($r = 0.72$) and between cell length and monosporangium length ($r = 0.75$; see also *fig. 1*), but no correlation between cell length and cell diameter ($r = 0.02$; see *fig. 2*).

Plants with single monosporangia have significantly longer cells and monosporangia than plants with clustered monosporangia, but the groups do not differ significantly in cell diameter (Mann-Whitney's U-test, $\alpha = 0.05$).

Within the group with clustered sporangia, plants with cylindrical stalk cells have significantly longer monosporangia than those with cup-shaped stalk cells; they do not differ significantly in either cell length or cell diameter (Mann-Whitney's U-test, $\alpha = 0.05$).

Implication of the positive relationships is that morphological characters are

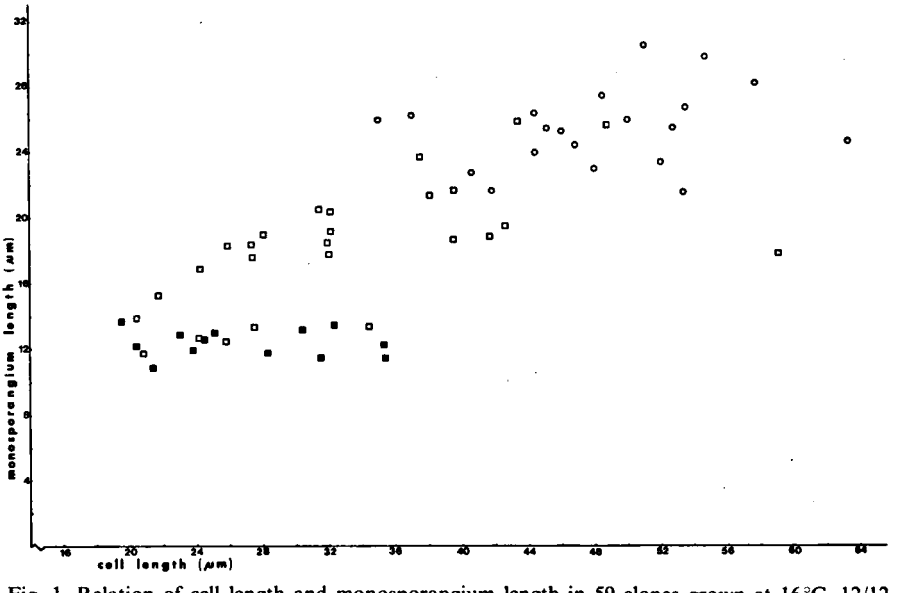


Fig. 1. Relation of cell length and monosporangium length in 59 clones grown at 16°C, 12/12 daylength. All measures averages of 5 plants. ○ = monosporangia single, □ = monosporangia clustered, ■ = monosporangia clustered, sporangial stalk cell cup-shaped.

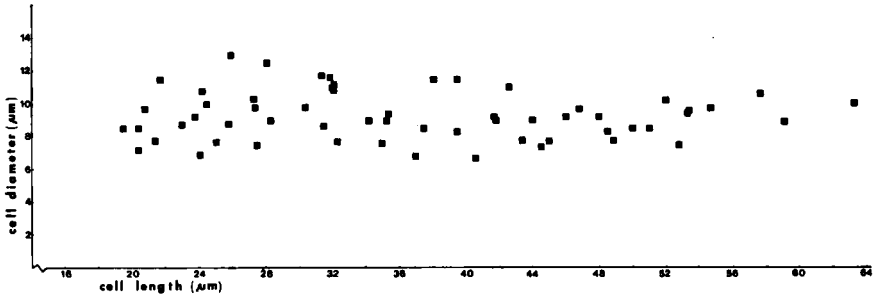


Fig. 2. Relation of cell length and cell diameter in 59 clones grown at 16 C, 12/12 daylength. All measures averages of 5 plants.

not randomly distributed over the whole range of clones. If, in addition to observing these coincidences in characters, we want to distinguish between different groups, we have to make a decision on which characters should be considered most important or most useful; since there is a certain continuity in all of the characters, the separation into any number of groups has a more or less arbitrary nature. From *fig. 1* we have concluded that in addition to the already mentioned characters of monosporangial arrangement and shape, cell length and monosporangial length can be used for the creation of three groups; each of these groups is defined in *table 2.*

The separation between groups I and II (monosporangia longer or shorter than 21 µm) largely coincides with the sporangia being single or clustered, but group I still contains some clones with clustered sporangia; these are isolates from *Mesogloia*; other clones from the same substrate and locality have been termed non-clustered, although the difference is one of degree (compare *figs. 5* and *6*).

The separation between groups II and III (monosporangia longer or shorter than 14 µm) largely coincides with monosporangial stalk cells being cylindrical or cup-shaped, but in group III some clones are found that have cylindrical stalk cells (compare *figs. 13* and *14*).

The effect of this division is that plants exhibiting either sporangia placed singly, or cup-shaped stalk cells, are always assigned to groups I and III re-

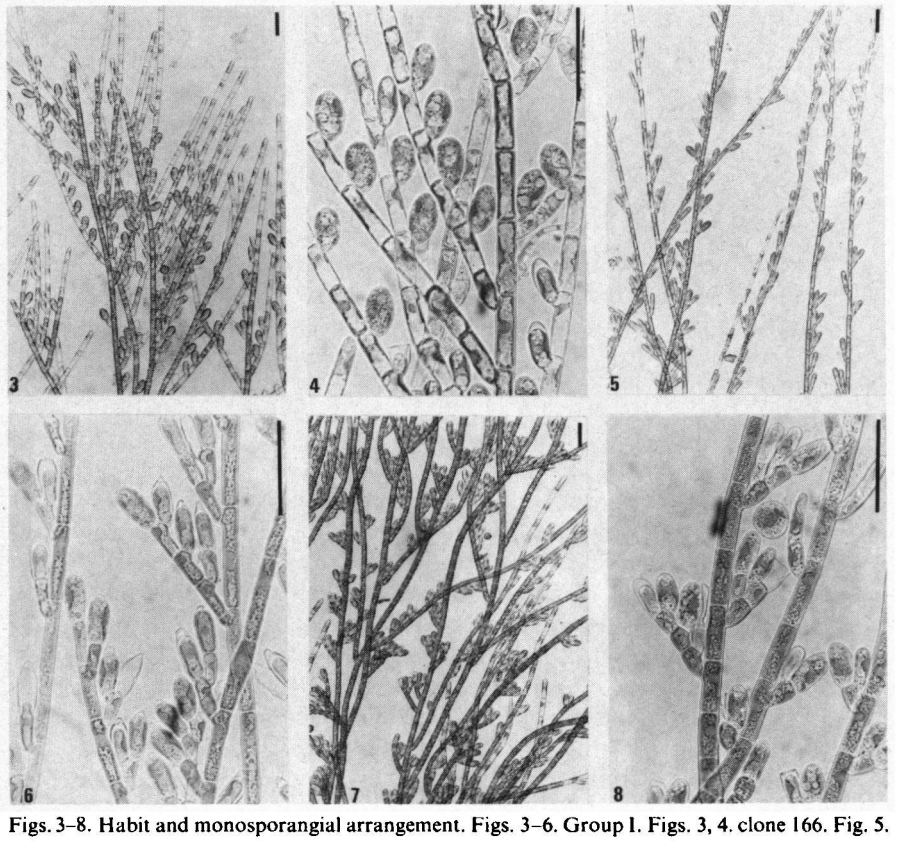
Table 2. Characteristics of 3 groups distinguished after growth of all 59 isolates under uniform conditions; see also *fig. 1*. All measures in µm.

	group I	group II	group III
cell length	35 – 65	21 – 44(60)	19 – 36
cell diameter	6.5 – 11.5	8.5 – 13.0	6.5 – 10.0
monosporangium length	21 – 32	15 – 22	11 – 14
monosporangium width	10.0 – 13.5	8.5 – 13.0	7.0 – 10.5
monosporangia clustered	– (+)	+	+
stalkcell cup-shaped	–	–	+(–)

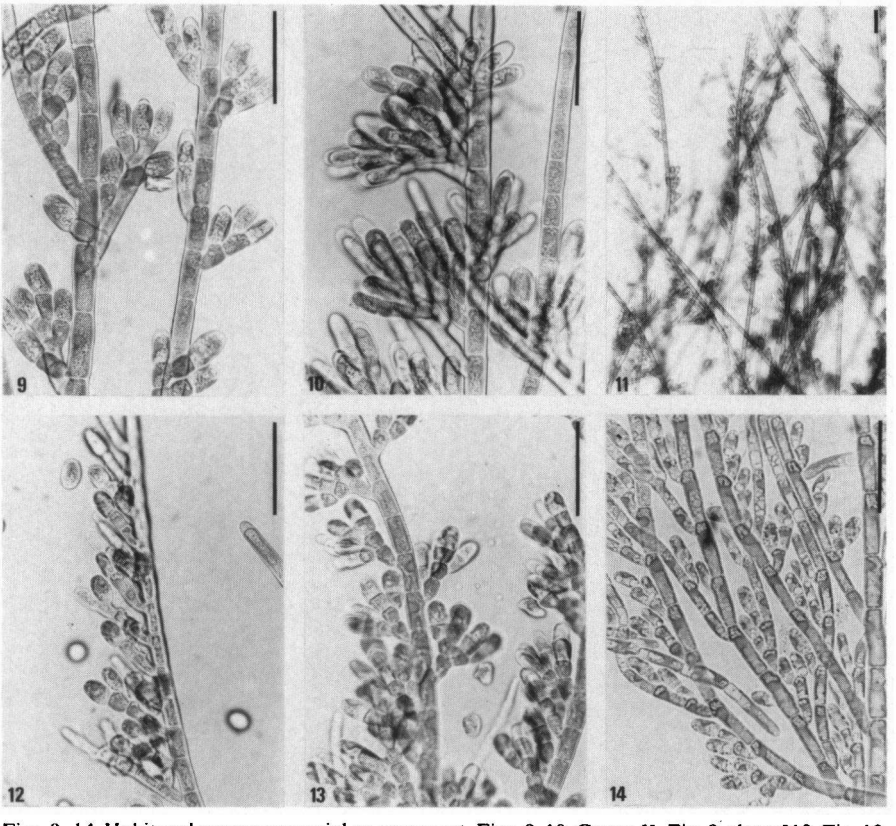
spectively, but all other plants are assigned to a certain group on the basis of their cell and sporangial dimensions. The significance of cell dimensions being used in the primary definition of these groups, is that they can be used for identification of sterile material, which often occurs in the field. It should be borne in mind, however, that cells may be somewhat shorter in the field than in culture. Largest cell diameters are found in group II, but there is an overlap with the other groups.

In the primary distinction of the three groups, which may be considered as species, no account is given of intraclonal variability; this will be done in chapter 3.2., and will necessitate some corrections of the definitions.

Group I (*figs. 3–6*) comprises the isolates from *Dasya baillouviana* and their culture-obtained tetrasporophytes (see STEGENGA & BORSJE 1976), as well as some tetrasporophytes isolated from various substrates in the Eastern Scheldt (Netherlands). Moreover, some Swedish isolates from *Mesogloia*, collected at 12–20 m depth, are included; the latter have shown no other reproduction than



Figs. 3–8. Habit and monosporangial arrangement. Figs. 3–6. Group I. Figs. 3, 4. clone 166. Fig. 5. clone 385. Fig. 6. clone 386. Figs. 7, 8. Group II. Fig. 7. clone 280. Fig. 8. clone 307. Scale bar in all figures 50 μm.



Figs. 9–14. Habit and monosporangial arrangement. Figs. 9, 10. Group II. Fig. 9. clone 312. Fig. 10. clone 316: Figs. 11–14. Group III. Fig. 11. clone 283. Fig. 12. Clone 284. Fig. 13. clone 309. Fig. 14. clone 507. Scale bar in all figures 50 μm.

by means of monosporangia. The localities of origin suggest this species to prefer sheltered conditions; tidal movement is absent from some of the collecting sites. It is of interest that gametophytes and tetrasporophytes of the same life history fall in the same group because of similar morphology, with the exception of the initial spore germination.

Group II (*figs. 7–10*) largely originates from the substrates *Nemalion* and *Codium*, where their prostrate parts usually become endophytic. The inclusion of a few clones from other substrates because of morphological similarity, suggests that this semi-endophytism is not a valuable systematic criterion. Among this group we found one clone (328) with sexual reproduction, which is described in more detail in chapter 3.3. No representatives of this species were collected on the Swedish west coast. Group II seems to prefer more exposed situations than group I.

Group III (*figs. 11–14*) is found on various substrates, but not usually forming an endophytic part. On tough substrates like *Cladophora* and *Corallina* it often

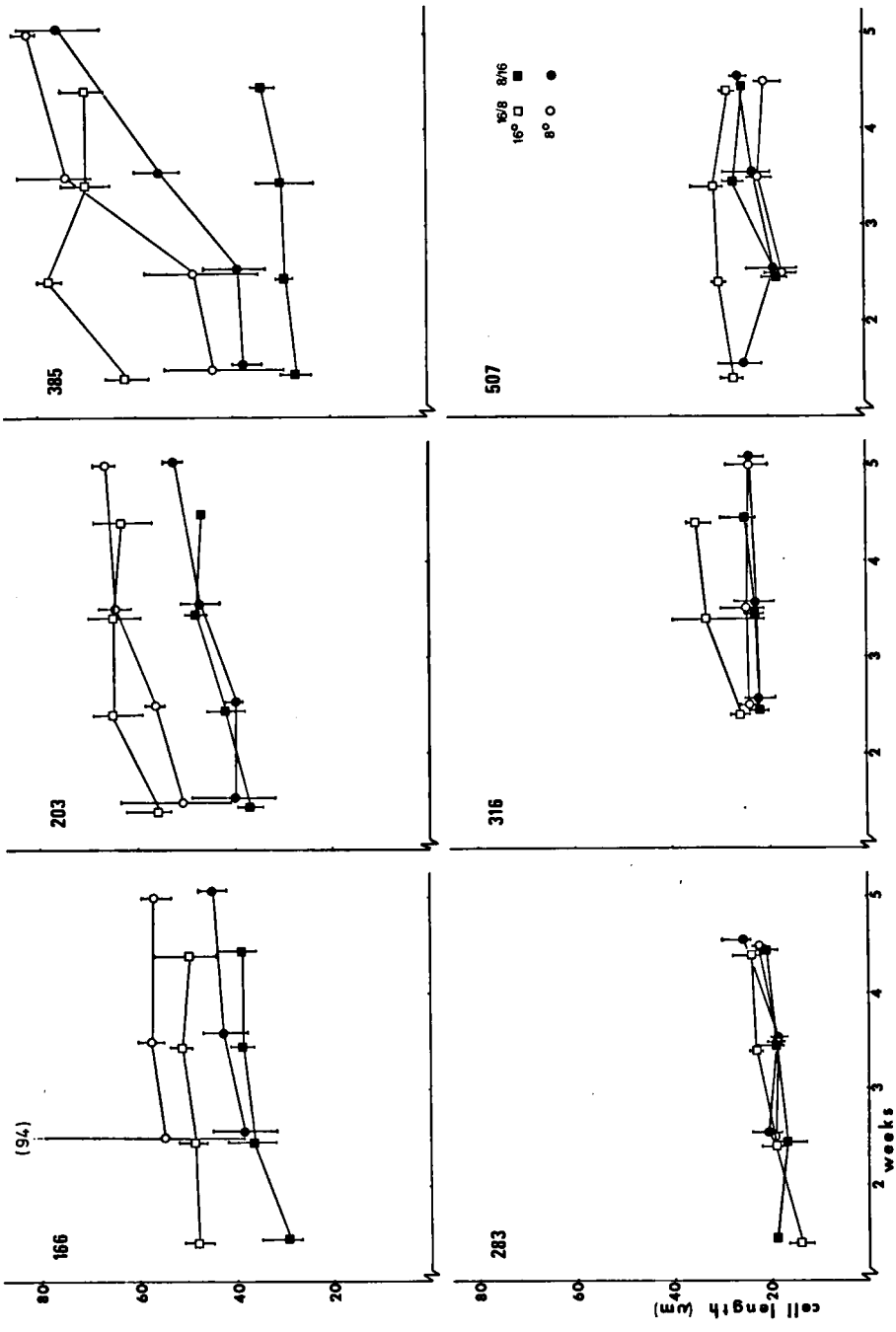


Fig. 15. Cell length in 6 clones grown at 8 and 16°C, 8/16 and 16/8 daylengths, measured at weekly intervals during c. 5 weeks. Averages and extremes of measurements on 5 plants.

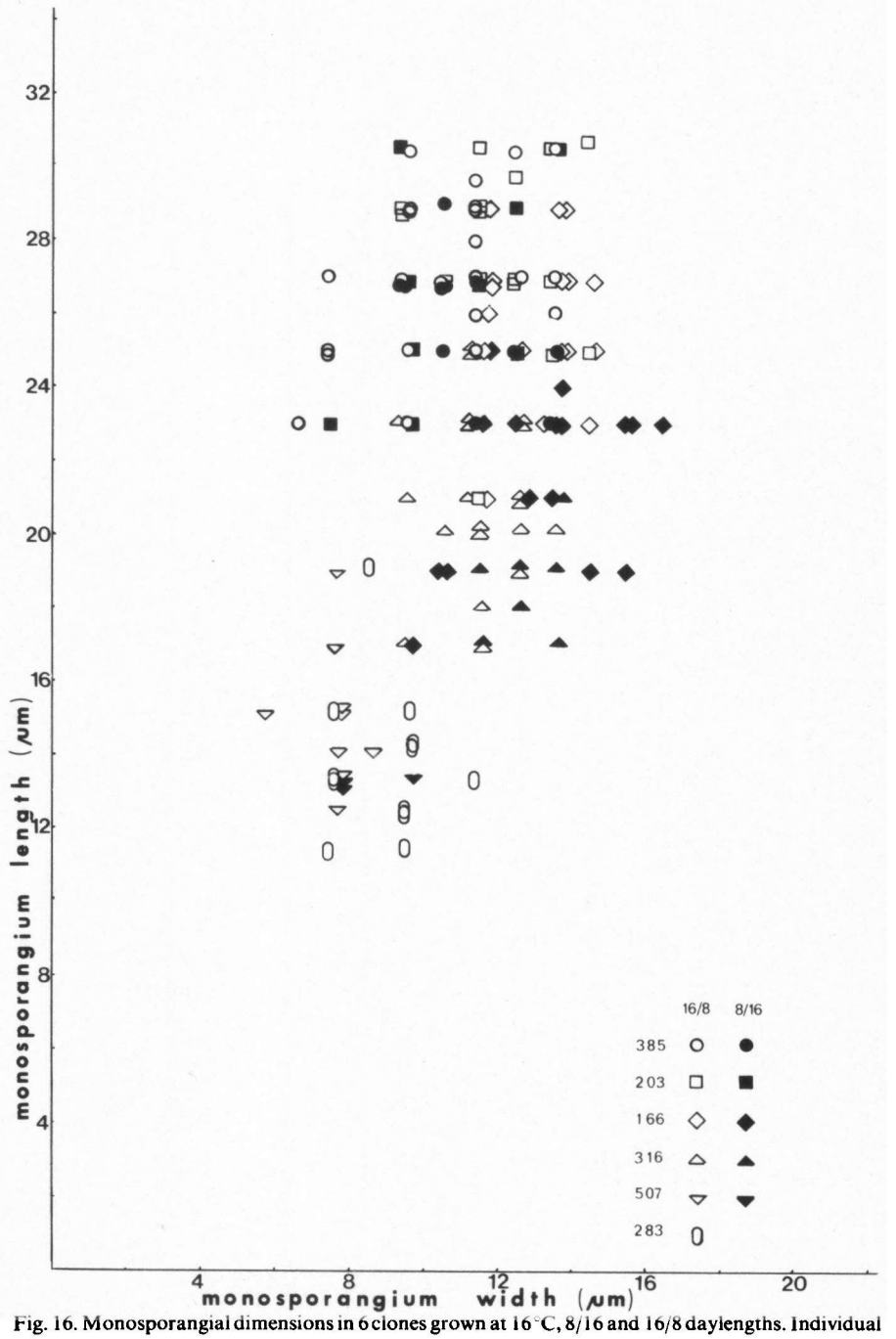


Fig. 16. Monosporangial dimensions in 6 clones grown at 16°C, 8/16 and 16/8 daylengths. Individual values.

forms tufts of many short-celled filaments, scarcely branched. In culture, neither sexual reproduction nor tetrasporangia have been found. This species, like group II, is found on the more exposed sites.

Comments on the identity of the three groups will be given later in this paper (chapter 4.1.).

3.2. Intraclonal variability

From the whole group of 59 isolates, Six representatives were chosen for studies on the intraclonal morphological variability, namely: 166, 203, 385 (group I); 316 (group II); 283, 507 (group III). Results are partly given in *figs. 15*, *16* and *17*, and will be briefly commented on here:

– Average cell length (*fig. 15*) was most variable in group I; in general, cells were longer under long day conditions, whereas temperature seemed to be of less influence. In groups II and III the effect of temperature and daylength was much less expressed or negligeable.

– In *fig. 16* individual measurements of monosporangial dimensions have been plotted, to show variation within clones in addition to variation between clones. Only data of 16 °C were used, since plants at 8 °C had usually not reached the reproductive state by the end of the experiment. On the average, monosporangia were a few μm shorter at short day than at long day; diameter of the sporangia was not significantly influenced. It appears that monosporangial lengths found in the first experiment (*fig. 1*) are on the lower side of the range of variation in the present experiment.

– Branching (*fig. 17*) again showed a characteristic difference between representatives of group I on one side, and groups II and III on the other. In group I degree of branching gradually increased with length of the main axis, and there was a significant influence of daylength, the highest number of laterals occurring at long day; the influence of temperature was not so much expressed. In groups II and III it is suggested that branching started at an earlier stage, namely when axes are 4–6 cells high as opposed to a *c.* 10–12 celled stage in group I. In groups II and III there is little evidence of branching intensity being linked to either temperature or daylength, òr ontogenetic stage of the plants; in these groups the degree of branching is partly determined by the number of monosporangial clusters on the main axes, and a separate check of indeterminate branches might present a different picture. Although each clone appears to have its own peculiar branching characteristics, which consistently differ between at least 2 groups, this character is either too much linked to external conditions or ontogenetic stage, or too variable to be used in species delimitation.

– Average cell diameter varied 2 or 3 μm in all clones, but was not clearly connected with conditions of temperature or daylength.

The variability patterns of cell length and branching confirm and emphasize the earlier found differences between group I on one side and groups II and III on the other. Differences between groups II and III appear not to be expressed in anything else than monosporangial dimensions and, partly, monosporangium shape.

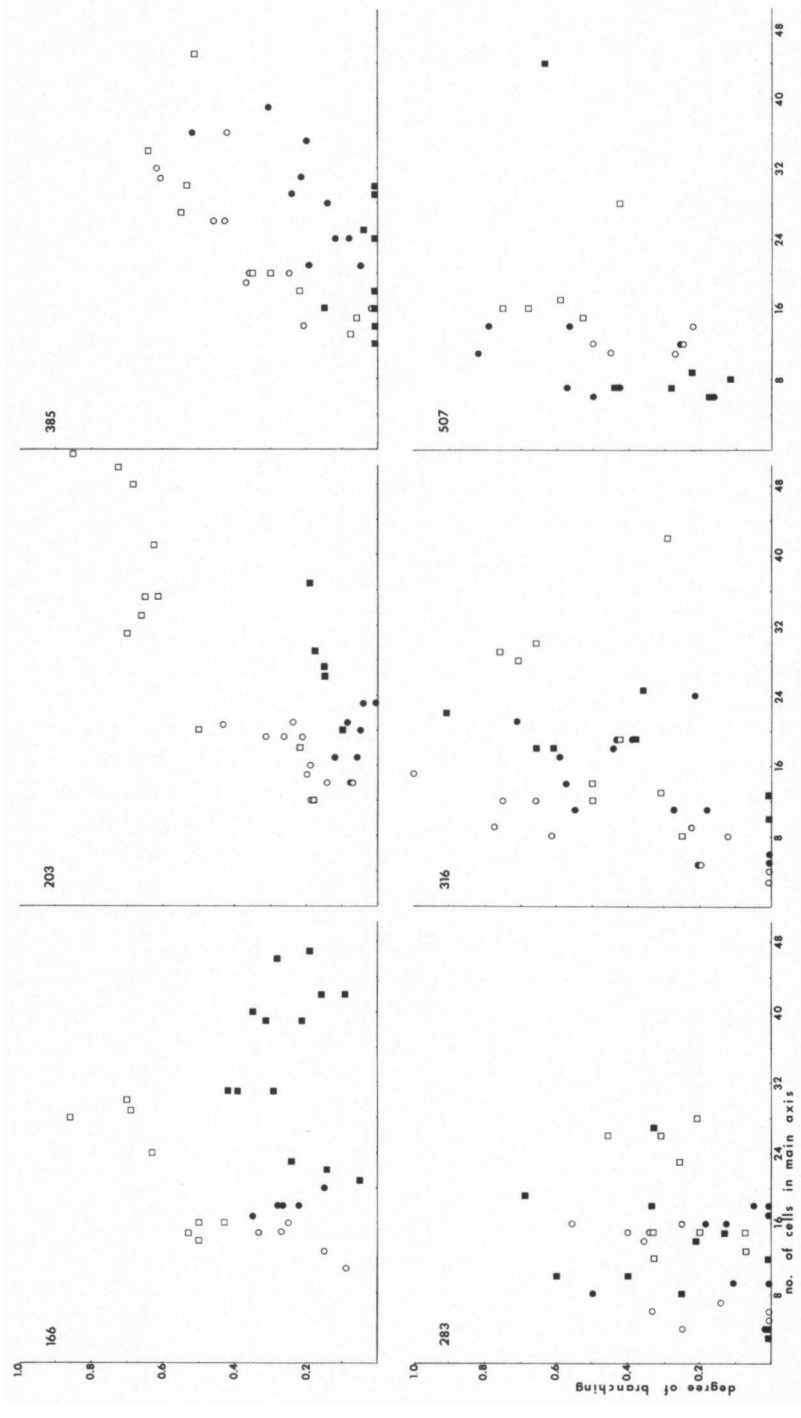


Fig. 17. Branching in relation to height of plant. Height as no. of cells in main axis; degree of branching as no. of laterals per main axis cell; symbols for different temperature and daylengths as in *fig. 15*. Individual values.

Considering this intraclonal variation, the definitions of the three groups have to be slightly readjusted in terms of cell dimensions (*table 3*, compare *table 2*). The range of cell length is wider in group I now, which at the lower extreme causes an overlap with groups II and III; it is mainly contributed by the short cells of clone 385 at 16 °C, short day. The overlap in monosporangial dimensions is larger; this is probably so because in *table 3* the extremes of individual measurements are used, whereas in *table 2* averages of measurements are given.

Table 3. Characteristics of the three groups after study of morphological variability of six clones; compare with *table 2*. All measures in μm.

	group I	group II	group III
cell length	25 – 85	20 – 43(60)	15 – 36
cell diameter	6.5 – 11.5	8.5 – 13.0	5.5 – 10.0
monosporangium length	19 – 31	15 – 25	11 – 19
monosporangium width	7.0 – 17.0	8.5 – 14.0	6.0 – 12.0
monosporangia clustered	– (+)	+	+
stalkcell cup-shaped	–	–	+ (–)

3.3. Life history

In a former paper (STEGENGA & BORSJE 1976) the life history was described of *A. dasyae*, a representative of group I; some clones of this species, gametophytes as well as tetrasporophytes, have been included again in the present investigation.

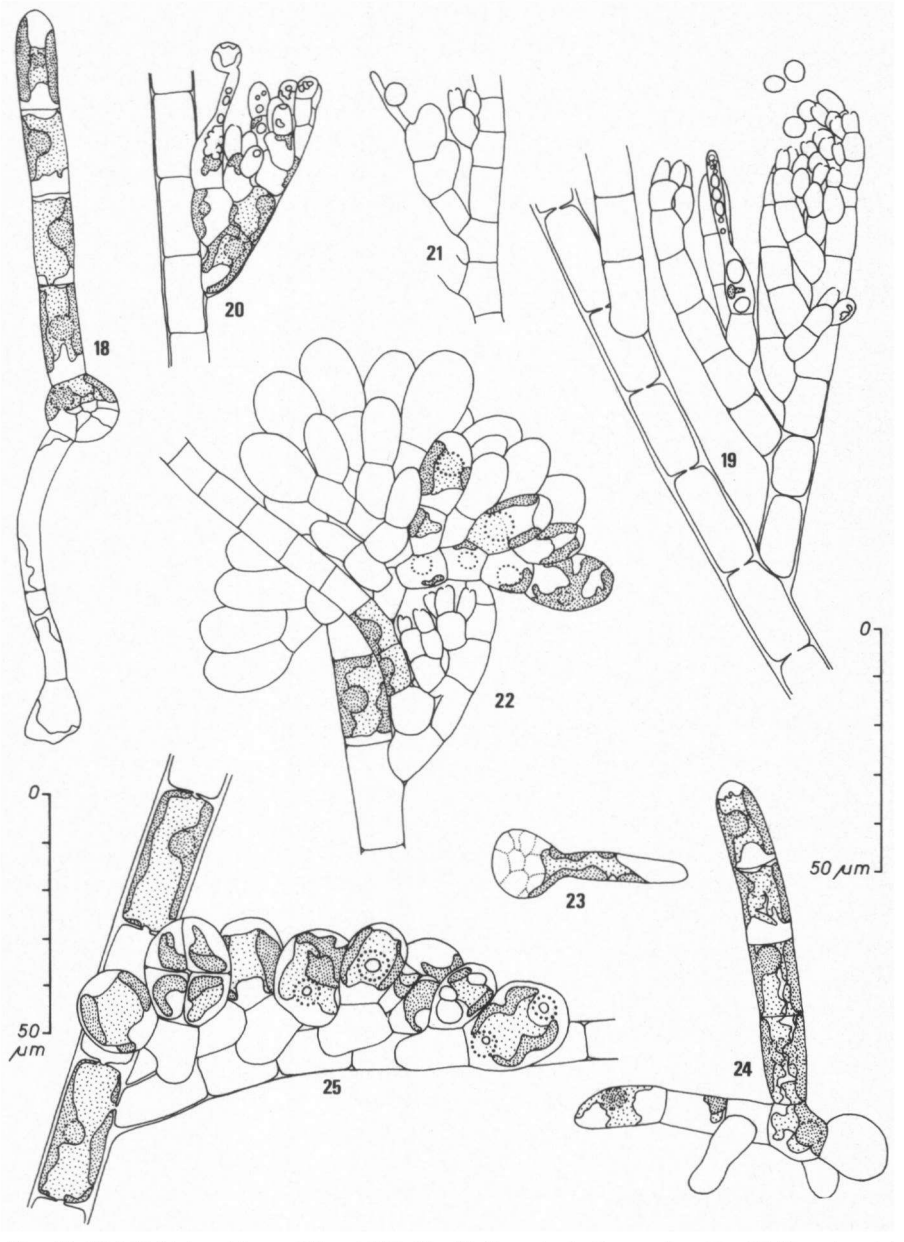
In addition we can now give some details of the life history of a representative of group II: clone 328 proved to be a bisexual gametophyte, and reproduced easily.

Germination of gametophytic monospores is bipolar; spores are persistent and form a prostrate filament on one side and an erect filament on the other (*fig. 18*); the spore remains globular in shape. The prostrate filaments develop into a multicellular filamentous base, which in turn may develop a number of secondary erect filaments. Erect filaments bear a number of indeterminate laterals and clusters of monosporangia or gametangia. Cells of the erect filaments measure (10)20–30 × 10–13 μm; monosporangia measure 16–19 × 11–12.5 μm.

Gametangia of both sexes are borne on much branched ramuli, the spermatangia being in the majority (*fig. 19*). Spermatangia measure 5–6 × 3.5–5 μm, carpogonia 15–17.5 × 6–7.5 μm, trichogynes are up to 38 μm long.

After fertilization (*fig. 20*) the carpogonium divides transversally (*fig. 21*) to form a small filament with numerous laterals. Finally, all terminal cells of the carposporophyte develop into carposporangia (*fig. 22*); mature carposporangia measure 17.5–20 × 11–14 μm. Mature carposporophytes might be confused with monosporangial clusters, but are larger in overall size and more globular in shape.

Carpospores and tetrasporophytic monospores germinate in a unipolar fashion (*fig. 23*), first forming a prostrate filament which develops into a multicellular base. Erect filaments are given off by this prostrate system (*fig. 24*). Cell



Figs. 18–25. Life history (clones 328 and 347). Fig. 18. Gametophytic germling. Fig. 19. Gametangial cluster. Fig. 20. Fertilized carpogonium. Fig. 21. Carpogonium after first division. Fig. 22. Mature carposporophyte with carposporangia. Figs. 23, 24. Tetrasporophytic germlings. Fig. 25. Tetrasporangia.

dimensions and branching do not differ significantly from the gametophyte. Monosporangia and tetrasporangia are borne in clusters. Monosporangia measure 16–22.5 × 11–14 μm, tetrasporangia (*fig. 25*) *c.* 23 × 20 μm, their division is cruciate. The tetrasporophyte was included earlier in this study as clone 347.

Environmental induction of sexual reproductive structures is summarized in *table 4*. In general it can be concluded that gametangia are formed after a certain

Table 4. Growth and reproduction of clone 328 (bisexual gametophyte) under different combinations of temperature and daylength. Given data are: maximum number of cells in main axis, and reproductive state; ○ = monosporangia, ♀ = carpogonia, ♂ = spermatangia, ⚲ = carposporophytes.

temperature and daylength		days after culture initiation					
		7	14	21	28	35	42
4°	8/16	0	0	0	discontinued	–	–
	16/8	0	4	5	7	10	12
8°	8/16	4	6	12	20	27	34
	16/8	6	9	17	20	25, ○	35, ○
12°	8/16	7	14	22, ♀	30, ○, ♀	32, ○, ♀, ♂	discontinued
	16/8	7	15	22, ○	28, ○, ♂	35, ○, ♂	discontinued
16°	8/16	8	16	24, ⚲, ♂	33, ○, ♀, ♂, ♀	?, ○, ♀, ♂, ⚲	discontinued
	16/8	12	24, ○	29, ○	40, ○	?, ○, ♀, ♂, ⚲	discontinued

ontogenetic stage, namely when the main axes are longer than 20 cells; a relatively high temperature is necessary, while short day apparently has a favourable effect on gametangium formation. A similar experiment with clone 347, the tetrasporophyte, was continued for 4 weeks, after which time only at 16°C, long day monosporangia and tetrasporangia in small quantities had been formed, while growth velocity was rather similar to that of the gametophyte. In earlier experiments we have observed tetrasporangia to be formed at 8°C and neutral day as well.

This species much resembles *A. dasyae* (STEGENGA & BORSJE 1976). Discriminating characters are the shape of the original spore after germination, the shorter cells, smaller sporangia of all kinds, and the asexual reproductive structures occurring in clusters. Sexual reproductive structures are much alike, except that *A. dasyae* is primarily dioecious, and its trichogynes are generally longer.

Clones 328 and 347 are included here in *A. nemalionis* (see discussion). Other representatives of this group have only reproduced by means of monospores. Germination morphology of these strains suggests them to belong to the tetrasporophytic part of this species.

4. DISCUSSION

4.1. Identification of the three groups

Three groups were distinguished in a number of 59 isolates. They may cover a

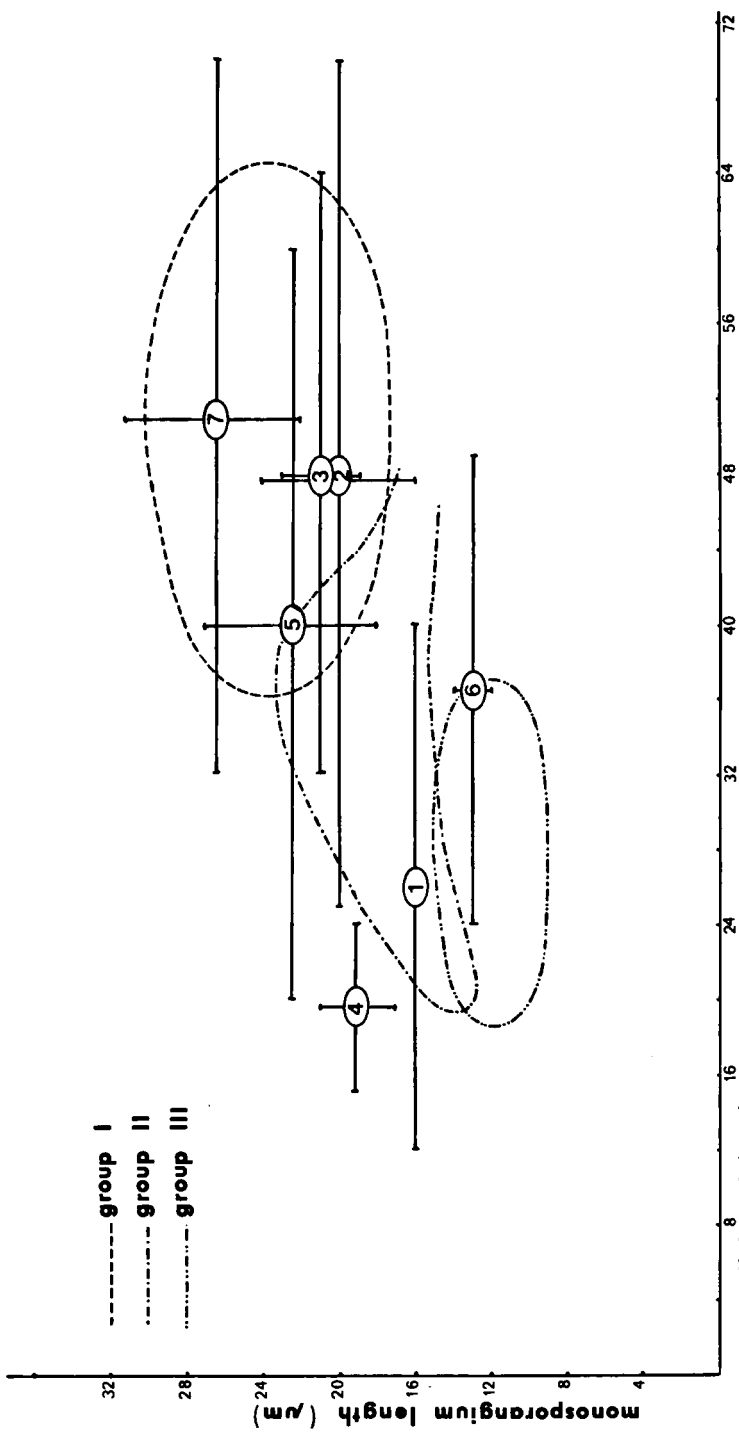


Fig. 26. Comparison of literature data with own results, with respect to cell length and monosporangial length; species with monosporangia placed singly. 1 = *A. dasyae* (COLLINS 1906). 2 = *A. dasyae* (WOELKERLING 1973). 3 = *A. intermedium* (JAO 1936). 4 = *A. savianum* (HAMEL 1928a). 5 = *A. savianum* (WOELKERLING 1973). 6 = *A. subtillissimum* (HAMEL 1928a). 7 = *A. zosterae* (= *A. subseriatum*; JAO 1936). Further explanation see text.

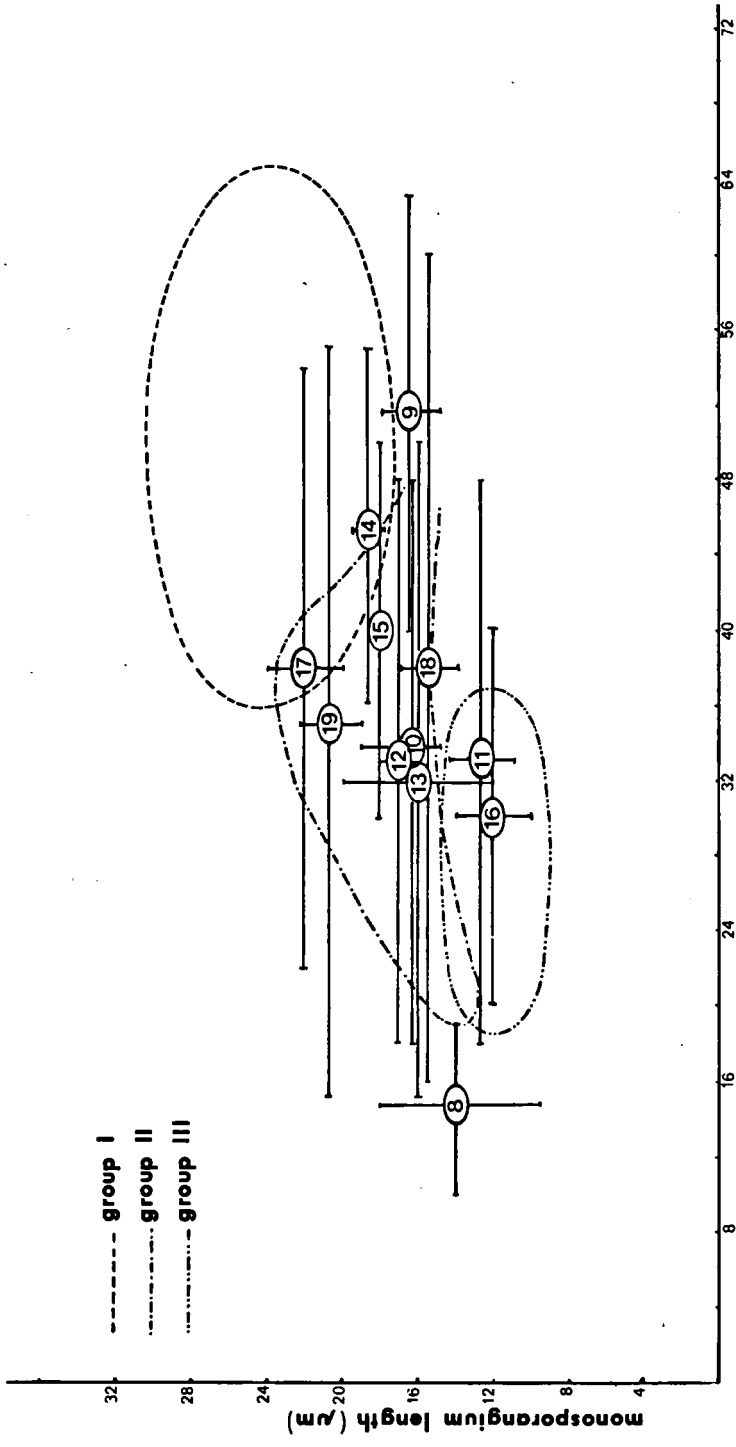


Fig. 27. Comparison of literature data with own results, with respect to cell length and monosporangial length; species with clustered monosporangia. 8 = *A. alcyonidiae* (JAO 1936). 9 = *A. corymbiferum* (HAMEL 1928a). 10 = *A. daviesii* (ROSENVINGE 1909 – pp.). 11 = *A. daviesii* (ROSENVINGE 1909 – pp.). 12 = *A. daviesii* (HAMEL 1928a). 13 = *A. daviesii* (WOELKERLING 1973). 14 = *A. nemalionis* (ROSENVINGE 1909). 15 = *A. nemalionis* (HAMEL 1928a). 16 = *A. penetrans* (LEVRING 1935). 17 = *A. thuretii* (BORNET 1904). 18 = *A. thuretii* (HAMEL 1928a). 19 = *A. thuretii* β *agama* (ROSENVINGE 1909). Further explanation see text.

large proportion of the variation within the epiphytic and semi-endophytic or semi-endozoic representatives of the genus *Acrochaetium* on the European Atlantic coast. Since no true gaps in any character exist between the three groups, their separation may appear somewhat artificial, and for reasons of convenience one might as well conclude to the conspecificity of this continuum of forms. Yet, we have preferred to give the groups specific status, first because the very large variation has been shown to be largely genetically determined and deserves some expression in systematic terms; secondly, in field work, detection of one or more of these species will be more meaningful than the distinction of only one undifferentiated species; we have shown that at least group I has an ecology different from that of the other groups, while group II has many semi-endophytic representatives as opposed to group III. On the other hand, distinction of more than 3 species would present substantial difficulties in identification.

Accepting the groups as species, we have attempted identification with the literature. A positive identification is not simple, partly because original descriptions of relevant species are not always complete with regard to cell dimensions and their variation (e.g. DILLWYN 1809), and partly because species descriptions have been altered in later work. In *figs. 26* and *27* a comparison is made between literature data and our results. This concerns the two measures found best applicable in species delimination, i.e. cell length and monosporangial length. The ranges of variation of cell length and monosporangial length are given by horizontal and vertical lines respectively, at the crossing giving the median value of each measure. If in the descriptions extremes in dimensions were given between brackets, these have not been included; in some species we had to calculate cell lengths from given cell diameters and length/diameter ratios; occasionally no measurements were given at all, and we have used whatever evidence we could obtain from figures. The three groups we have distinguished in the present study are circumscribed by the various broken lines; their extent is immediately derived from the results represented in *fig. 1*.

Group I agrees quite well with *A. zosterae* Papenfuss (= *A. subseriatum* Jao) and *A. intermedium* Jao; also *A. dasyae* and *A. savianum*, as interpreted by WOELKERLING (1973) are satisfactory, but the original description of *A. dasyae* as presented by COLLINS (1906) and HAMEL's (1928a) description of *A. savianum*, based on original material, reported much smaller cell dimensions. *A. subtilissimum*, another species reported to possess monosporangia in single arrangement, is of a much smaller size as regards its cell dimensions. As for the species with more or less clustered sporangia, the range of variation of two of them extends well into group I; they are *A. thuretii*, the original figures of BORNET (1904) as well as ROSENVINGE's (1909) description of β agama, and ROSENVINGE's (1909) account of *A. nemalionis*. It is of interest that these are about the only European records in agreement with group I. HAMEL's (1928a) account of *A. thuretii* differs from our interpretation with regard to sporangial dimensions, and is more in agreement with group II; this also holds true for other records of *A. nemalionis*. For reasons of convenience we have kept the name *A. zosterae* for this species. this being the first name accompanied by an adequate and non-

challenged description completely in agreement with group I. It seems likely, however, that some of the above mentioned species are to be accepted as synonyms; if so, the name *A. savianum* has priority (WOELKERLING 1973).

Group II agrees with some descriptions of *A. daviesii* (HAMEL 1928a, WOELKERLING 1973), and with most accounts of *A. nemalionis*; since a number of clones in group II were isolated from *Nemalion*, conspecificity with *A. nemalionis* is almost certain. This species is also found on *Codium*, where it forms an endophytic part similar to that in *Nemalion*. A confusion with *A. codii* in some reports is therefore not to be excluded (e.g. LEVRING 1937; DEN HARTOG 1959 – *A. codii* is probably absent from the Dutch coast); *A. codii* has much larger cell dimensions, and generally more than one chloroplast per cell, and although a member of the genus *Acrochaetium*, it does not belong to any of the groups under consideration in the present paper. Obviously, most epiphytic representatives of this group have in the past been referred to *A. daviesii*, like our group II a species commonly met with in the field. From DILLWYN'S (1809) description it is not clear whether the name *A. daviesii* should be attached to our group II or III. As regards monosporangial dimensions, HAMEL's (1928a) descriptions of *A. corymbiferum* and *A. thuretii* belong to this group. Provisionally we have retained the name *A. nemalionis* for this group.

Group III agrees perfectly with ROSENVINGE's (1909) account of *A. daviesii*, be it only part of the description. Rosenvinge made mention of two distinct groups in *A. daviesii*, distinguished on the length of the monosporangia; the shorter sporangia compare well with our group III, the longer ones have more affinity with group II; like in our work (see *table 2*) the separation lies between 14 and 15 μm. No description mentions explicitly the curious shape of monosporangium and monosporangial stalk cell, found in many representatives of group III; it was clearly depicted by KORNMANN & SAHLING (1977), but may also be responsible for JAO's (1936) record of bisporangia in *A. alcyonidiae*. As regards cell dimensions, *A. alcyonidiae* is rather on the small side, and it is not sure whether it is a semi-endozoic form of group III. *A. penetrans* (LEVRING 1935), another semi-endozoic species, belongs to group III with more certainty; its cell and monosporangial dimensions are in perfect agreement. Recent accounts of *A. daviesii* by WOELKERLING (1973) and DIXON & IRVINE (1977) apparently include representatives of our groups II and III. The scarce data on *A. sparsum* (e.g. DIXON & IRVINE 1977) suggest a position in group II or III as regards sporangial arrangement, but given dimensions of sporangia are in agreement with neither group. Despite the lack of sufficient positive evidence, we have kept the name *A. daviesii* for group III.

Identification with the literature has been limited to a few publications concerning the North Atlantic; on world scale there are many potential synonyms; comparison of these seems only useful if material of relevant areas is available.

4.2. Delimitation of the genus *Acrochaetium*.

The generic name *Acrochaetium* was created by NÄGELI (1861); the genus was define as containing (acrochaetioid) algae that reproduced by means of

"Schwärmsporen", released from "Sporenmutterzellen"; apparently structures were meant which nowadays are called monospores and monosporangia. By this character it was distinguished from *Rhodochorton* Nägeli, whose reproduction was said to take place by means of tetraspores. Together the genera *Acrochaetium* and *Rhodochorton* comprised the marine representatives of the acrochaetioid algae, which formerly had been included in various other genera, often together with other small filamentous algae of different systematic affinities. A more detailed history of nomenclature is given by DREW (1928) and WOELKERLING (1971).

NÄGELI's (1861) definition of *Acrochaetium* and *Rhodochorton* has been adopted by several authors (BØRGESEN 1927, HAMEL 1928a, b, ABBOTT 1962, AZIZ 1965), and it is still used in this sense in some recent floras (ABBOTT & HOLLENBERG 1976, RUENESS 1977). The interpretation has slightly altered, however: NÄGELI (1861) proposed to assign *Callithamnion virgatulum* and *Callithamnion daviesii* to *Rhodochorton*, should possession of tetraspores be proved in these species. Later authors generally included in *Rhodochorton* only forms that bear exclusively tetrasporangia, whereas species of *Acrochaetium* may form tetraspores in addition to monospores. The genus *Acrochaetium* thus contained the majority of species with acrochaetioid morphology. A few newly-established genera, e.g. *Colaconema* (BATTERS 1896) and *Kylinia* (ROSENVINGE 1909) included only a small number of species, since they were based on rather unique peculiarities.

PAPENFUSS (1945) placed in *Acrochaetium* only species with parietal chloroplasts, whereas species with stellate chloroplasts were united in *Chromastrum* (later *Kylinia* – PAPENFUSS 1947).

KYLIN (1956) used basal structure as an important criterion and assigned to *Acrochaetium* the species with multicellular bases and 1(–2) chloroplasts per cell, stellate or parietal, with or without pyrenoids; another important genus was *Kylinia*, in this case interpreted to include all species with unicellular base. The same systematic arrangement was recently still followed by BOLD & WYNNE (1978).

FELDMANN (1962) included in *Acrochaetium* most species with a supposedly haplobiontic life history, and one chloroplast per cell; sexual reproductive structures distinguished *Acrochaetium* from a few smaller genera with the same chromatophore characters. In effect, Feldmann's *Acrochaetium* would include most of Kylin's *Acrochaetium* and *Kylinia*, or Papenfuss's *Acrochaetium* and *Chromastrum*, and thus again the majority of the acrochaetioid algae.

In systematic revisions, WOELKERLING (1971) and DIXON (in PARKE & DIXON 1976) have discarded the name *Acrochaetium*, and accepted *Audouinella* as the most important or only generic name in this group of algae.

STEGENGA & VROMAN (1977) preliminary proposed a system in which the family was split into 5(–7) genera, defined by a combination of morphological and life history characters. The resulting system showed most resemblance to that of PAPENFUSS (1945), since it was found that a certain chromatophore structure coincides with a special type of life history and morphology of alternat-

ing phases. In this context, the genus *Acrochaetium* was reestablished for species with one, occasionally a few parietal chromatophores, each provided with a pyrenoid; the basal part is multicellular filamentous in both free-living generations; spore germination is unipolar or bipolar; the carposporophyte is well developed and produces many carpospores.

4.3. Comparison of the studied species with other Acrochaetiaceae.

The present study presumably treats a large section of the genus *Acrochaetium* as it occurs on the Western European coast, namely the species with well developed erect parts and one chloroplast per cell. Not included was *A. codii*, a species with very large cell diameters, known from the French Atlantic coast; it occurs on *Codium* and *Nemalion*, and in that case has an endophytic base, but also on other substrates (e.g. *Cystoseira*) and is then completely epiphytic. This species has a few chloroplasts per cell, each with a pyrenoid, and in some respect is reminiscent of *Rhodochorton floridulum*. It is probably conspecific with *A. botryocarpum*, which species apparently has a life history of the *Acrochaetium* type (WOELKERLING 1970). The plant found on the European coast has formed tetrasporangia in culture, but tetraspores have failed to germinate (Stegenga, unpublished observation). Relationship to *A. caespitosum* and *A. lorrain-smithiae* is suggested by similar habit and cell dimensions, but the alleged absence of pyrenoids or even complete lack of knowledge on chloroplast structure in these species, makes comparison difficult (cf. HAMEL 1928a; DIXON & IRVINE 1977).

An endophytic species, namely *A. asparagopsis* (MAGNE 1977 – as *A. asparagopsidis*) positively belongs to *Acrochaetium*. Besides the typical phases in the life history, it also has the capacity to form scarce erect filaments, at least in culture; the latter bear monosporangia, in shape and arrangement reminiscent of *A. daviesii*. Species like *A. asparagopsis* may indicate a reductional trend in the genus *Acrochaetium*, leading to endophytic and endozoic species. The reduction may affect the erect parts of gametophytes as well as tetrasporophytes. In the meantime, this does not imply that all endophytic or endozoic species of acrochaetioid algae are in the same way related to the genus *Acrochaetium* as defined herein; a similar reductional trend in the genera *Audouinella*, *Chromastrum* and *Rhodochorton* must be held possible as well. It is assumed that characteristics of the chloroplasts are of primary importance in deciding about these relationships.

Likewise, a reduction affecting the prostrate part of *Acrochaetium* species, may lead to plants with a unicellular base, which we have earlier assigned to the genus *Kylinia* (STEGENGA & VAN WISSEN 1979). That this reduction should apparently only concern the gametophytic generation, may be explained by the difference in spore germination between the two generations of some *Acrochaetium* species: in *A. dasyae* (STEGENGA & BORSJE 1976) and *A. nemalionis* (the present communication) the gametophytes have a bipolar germination from a persistent spore, whereas the tetrasporophytes basically have a unipolar germination pattern, forming a prostrate filament first, usually under loss of spore contents. A complete reduction affecting the prostrate part of the tetraspor-

ophyte, would thus necessarily eliminate the whole generation. Since information on germination patterns in *Acrochaetium* is scanty at this moment, some caution is needed on this hypothesis, however.

In conclusion, it is assumed that the life history of *Acrochaetium* species with epiphytic isomorphic generations represents a basic type, and that some endophytic and endozoic species, the genus *Kylinia*, and possibly also *Rhodochorton floridulum* can be derived from it by reduction of various parts. Another reductional trend frequently found, is the loss of capacity to form sexual reproductive organs (including tetrasporangia). This has not necessarily been accompanied by morphological changes, so that some morphological entities have sexual as well as asexual clones; the very effective asexual reproduction by means of monospores has probably facilitated the prolonged existence of the latter forms. We do not follow the suggestion of WOELKERLING (1971) to unite the asexual species in a separate genus *Colaconema*.

In the whole of the family Acrochaetiaceae, the genus *Chromastrum* appears to take a place somewhat apart from the other genera, i.e. we have no knowledge of species that could be regarded as true intermediates between *Chromastrum* and the other genera. Consistent differences are chromatophore structure and spore germination in the tetrasporophyte. The other genera (*Audouinella*, *Acrochaetium*, *Kylinia* and *Rhodochorton*) are more related to each other: chloroplasts are parietal in all of them, and unipolar germination with loss of spore contents is a common feature among their tetrasporophytes. The genus *Rhodochorton* takes a somewhat isolated position however, because of deviating postfertilization developments, so that germination patterns of tetrasporophytes are not relevant. At present we have no insight in the importance of presence or absence of pyrenoids in determining systematic relationships; pyrenoids are absent from *Audouinella* and all representatives of *Rhodochorton* except *R. floridulum*.

STEGENGA & MULDER (1979) have shown the main distribution of the genus *Chromastrum* to lie in the temperate and cold waters, particularly those of the North Atlantic Ocean. This is expressed in absolute as well as in relative numbers of recorded species. Hence, it may be inferred, that *Acrochaetium*, being the largest genus in terms of described species, takes a more prominent place and is more diversified in warm areas; actually, large numbers of species have been described from such areas (e.g. BØRGESEN 1915–1920). For this reason it may be argued that the species under consideration in the present paper do not represent a good cross section of the genus, and speculations on the effect of a revision in the genus *Acrochaetium* with respect to geographical distribution are better left behind until more experimental work has been done in tropical and subtropical areas.

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