LIFE-HISTORY STUDIES ON RHODOPHYCEAE II. HALYMENIA FLORESIA (CLEM.) AG.

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SUMMARY

Carpospores isolated from *Halymenia floresia* gametophytes collected at Banyuls (France, Pyrénées Orientales) grew into *Acrochaetium*-like plants. This *Acrochaetium* phase was capable of reproduction by monospores. Some of the *Acrochaetium*-like plants thus obtained differentiated into *Halymenia* sporophytes that produced monospores from monosporangial sori, and no tetrasporangia. These monospores developed into *Acrochaetium*-like or disciform plants, from some of which differentiated new *Halymenia* gametophytes.

Carpospores isolated from *Halymenia floresia* gametophytes collected at Rovinj (Yugoslavia) grew into *Acrochaetium*-like plants. This *Acrochaetium* phase was capable of reproduction by monospores. Some of the *Acrochaetium*-like plants thus obtained differentiated into *Halymenia* plants that reproduced firstly by means of gemmae and secondly by the formation of "apical pompons" from necrotic apices. "Apical pompons" were *Acrochaetium*-like plants grown from islands of living cortical cells lying amidst necrotic tissues. No new gametophytic *Halymenia* plants were obtained in cultures of Rovinj material, and therefore in culture the life-history was not completed.

Vegetative isolates (from cortical filaments) of Rovinj gametophytes grew into *Acrochaetium*-like plants reproducing by monosporulation. These *Acrochaetium* plants were not able to redifferentiate into *Halymenia* plants. Three types of such gametophytic *Acrochaetium* phases, differing in growth form and intensity of monosporulation, could be distinguished.

1. INTRODUCTION

In the first paper of this series our observations on the strikingly heteromorphic life-history of *Acrosymphyton purpuriferum* (*Cryptonemiales*, *Dumontiaceae*) were reported. Until recently only a summer annual gametophytic phase was known for *Acrosymphyton*. We gave evidence for the existence of a creeping, *Hymenoclonium*-like tetrasporophytic phase, which most probably ensures hibernation of this species.

In the present paper our observations on the isomorphic life-history of *Halymenia floresia* (*Cryptonemiales*, *Grateloupiaceae*) are treated. For *Halymenia floresia*, as it occurs in the Mediterranean, summer annual gametophytic as well as sporophytic phases were known (*cf.* FUNK 1955; KYLIN 1956; FELDMANN 1942). These two phases had only been collected during the summer half-year and it was not known in which form *Halymenia floresia* could hibernate in the Mediterranean.

2. MATERIAL AND METHODS

Unialgal cultures of *H. floresia* were isolated from carposporic plants collected at Rovinj, Yugoslavia (August 1967, from a depth of a few m, collector Mr. H. Rietema) and from carposporic plants collected near Banyuls, Pyrénées Orientales, France (September 1967, dredged from a depth of 30–40 m near Cap l'Abeille).

Unialgal cultures were started from isolated determinate laterals (Rovinj material) and from isolated carpospores (Rovinj material and Banyuls material).

The cultures were grown in a modified Erdschreiber-medium. Most of them were kept in a 17° (±1°) C culture room and exposed to light intensities of ca 1000–1500 lux for 16 hour photoperiods daily.

Series of cultures were then subjected to standard experimental conditions to test the possible combined influence of daylength and temperature on life-history and morphogenesis. For a more detailed treatment of these methods, see CORTEL-BREEMAN and VAN DEN HOEK (1970).

3. RESULTS

3.1. New Halymenia plants grown from carpospores

After about four months *Halymenia* carpospores (from both Rovinj and Banyuls material) had grown into *Acrochaetium*-like dark red pompons up to 1 mm in diameter. These pompons were each cultured separately in a series of 18 culture tubes, twelve of which received Rovinj material while the other six received Banyuls material. Morphologically, these *Acrochaetium*-like pompons were similar to those grown from vegetative isolates from Rovinj gametophytes (*cf. figs. 42, 43*; 3.2).

3.1.1. New Halymenia plants grown from carpospores isolated from Rovinj gametophytes

One month after the distribution of 12 filamentous pompons over 12 culture tubes the pompons started to differentiate into young *Halymenia* plants (*fig. 1*). These *Halymenia* plants were presumed to be tetrasporophytes.

In two culture tubes dissemination of the pompons, most probably by monospores, had taken place, as a result of which one tube contained two pompons, another tube five pompons. In the course of time all pompons differentiated into *Halymenia* plants. For some of them, however, it took several months before differentiation started. In the meantime such "retarded" pompons increased in size.

About two months after the distribution of the pompons into 12 culture tubes the young *Halymenia* plants were 2 to 4 mm high; after three months 6 to 11 mm high, after seven months $2\frac{1}{2}$ to $3\frac{1}{2}$ cm high (*figs. 2–4, 5–8, 44, 45*). At this stage the *Halymenia* plants were transferred from the culture tubes into 10 Erlenmeyer bottles containing 300 ml culture fluid. As the *Halymenia*

plants were possibly tetrasporophytes they were searched for tetrasporangia, but these could not be found. Many tips of the *Halymenia* plants at this stage appeared to bear pompons of *Acrochaetium*-like filaments (*figs. 44, 45, 9, 10, 11*). These pompons – termed *apical pompons* to distinguish them from basal pompons – appeared to sprout from islands of living cells lying amidst necrotic tissues.

3.1.1.1. Regenerative and reproductive capacity of apical pompons and apical *Halymenia* fragments

In order to test the regenerative capacity of apical pompons and of vegetative fragments 10 apical pompons were transferred each into one of ten culture tubes; and 10 apical fragments of *Halymenia* plants were transferred each into one of ten culture tubes.

In the course of the next three months it appeared that:

1. apical pompons were able to reproduce by monospores,
2. apical pompons and their monosporic offspring were able to differentiate into new *Halymenia* plants,
3. apical *Halymenia* fragments continued their growth and developed into *Halymenia* plants.

It should be noted, however, that only a limited number of the new pompons (grown from monospores produced by apical pompons) gave rise to new *Halymenia* plants, namely out of a total number of ca 230 new pompons only four had given rise to new *Halymenia* plants (the number of new pompons per culture tube varied from 5 to 120).

3.1.1.2. Regenerative and reproductive capacity of apical pompons, basal pompons, and apical *Halymenia* fragments

In view of the fact that only such a limited number of apical pompons and their offspring differentiated into *Halymenia* plants the question arose as to whether basal pompons would differentiate more easily into *Halymenia* plants than apical pompons.

Therefore the following comparative cultures in 40 ml deep petri dishes containing 30 ml culture fluid were set up:

6 apical pompons were transferred each into one of six petri dishes;
6 basal pompons were transferred each into one of six petri dishes;
2 apical *Halymenia* fragments were transferred each into one of two petri dishes;
9 pompons from vegetative isolates of *Halymenia* gametophytes (Rovinj material, *cf.* 2. and 3.2.) were transferred each into one of nine petri dishes, for comparison.

In the course of the following two months it appeared that:

1. basal as well as apical pompons were able to reproduce by monospores, as were the pompons grown from vegetative gametophytic isolates;
2. basal as well as apical pompons and their monosporic offspring were able to differentiate into *Halymenia* plants; this in contrast to pompons from vegetative gametophytic isolates and their offspring which never differentiated into *Halymenia* plants;

3. apical *Halymenia* fragments continued their growth and developed into *Halymenia* plants.

Of a total number of ca 235 pompons (diam. 1½–4 mm) derived from apical pompons only 5 had developed into young *Halymenia* plants.

Of a total number of 151 pompons (diam. ½–3 mm) derived from basal pompons 18 had developed into young *Halymenia* plants.

These results suggested that basal pompons and their monosporic offspring were better able to develop into *Halymenia* plants than apical pompons and their offspring. However, the difference is not very striking.

After eight months the monosporic offspring from basal as well as apical pompons had grown into partly interwoven felty masses of pompons, part of which were floating at the surface of the culture fluid. The *Halymenia* plants (up to 1½ cm high) differentiated from some of the young pompons did not bear tetrasporangia or sexual reproductive structures.

After eight months the apical fragments of *Halymenia* plants had grown into *Halymenia* plants up to 4 cm high and bearing apical pompons sprouting from islands of living cells amidst necrotic tissues; these *Halymenia* plants did not bear tetrasprangia or sexual reproductive structures.

3.1.1.3. Gemmae; an additional means of vegetative reproduction

The 10 *Halymenia* plants grown during 7 months in culture tubes and subsequently during 4 months in Erlenmeyer bottles (see 3.1.1.) containing 300 ml culture fluid did not increase very much in size. The maximum size reached in culture amounted to about 4½ cm.

In these older cultures the *Halymenia* plants appeared to have an additional means of vegetative propagation, namely by "gemmae". Gemmae were formed terminally on long-celled filaments growing from the inner cortex to the surface of the thallus. A gemma consisted of a clump of spherical cells (*fig. 12*). Gemmae were grouped together in sori (*fig. 46*).

3.1.1.4. Attempts to induce formation of tetrasporangia

The *Halymenia* plants grown from carpospores were possibly tetrasporophytes. However, although they were regularly searched for tetrasporangia these were never found. In an attempt to induce the tetrasporangia 6 *Halymenia* plants (2½–4½ cm high) were transferred to 12 °C short day conditions. In the course of 7 months following transfer no tetrasporangia were observed. Also *Halymenia* plants and basal pompons were subjected to the standard experiment to test the combined influence of daylength and temperature (see CORTEL-BREEMAN & VAN DEN HOEK 1969). The results were negative; no tetrasporangia were obtained. It was interesting, however, that 4 °C long day as well as 4 °C short day cultures died. Further, under 12 °C long day and 12 °C short day conditions *Halymenia* plants showed some growth; the basal pompons showed dissemination under 12 °C short day as well as 12 °C long day conditions. However, only under 12 °C long day conditions did some of the pompons develop *Halymenia* plants, and not so under 12 °C short day conditions.

3.1.2. New *Halymenia* plants grown from carpospores isolated from Banyuls gametophytes

About two months after distribution over 6 culture tubes five of the six pompons had started to differentiate into *Halymenia* plants. One culture tube contained, apart from a *Halymenia* plant, 9 new pompons. After about six months five culture tubes contained *Halymenia* plants (1½-3 cm high) and one culture tube only one pompon (2 mm diam.).

At this stage the cultures were transferred each into one of six Erlenmeyer bottles containing 300 ml culture fluid.

During the six subsequent months in Erlenmeyer bottles the *Halymenia* plants reached sizes of 2½-3 cm. As the *Halymenia* plants were possibly tetrasporophytes, they were searched for tetrasporangia, but these could not be found.

However, these *Halymenia* plants appeared to reproduce vigorously by monospores formed in monosporangial sori on the surface of the *Halymenia* thallus (*figs. 13–15*). In the end almost the whole surface of a *Halymenia* plant could be covered by monosporangial sori. Such plants had a characteristically contorted, more or less warty surface (*fig. 16*).

In the sori the outermost cortex-cells were monosporangium mother cells which cut off monosporangia by oblique walls (*fig. 13–15*).

The monospores produced by the *Halymenia* thallus grew into *Acrochaetium*-like pompons. This type of reproduction was much more intensive than that observed in *Halymenia* cultures of Rovinj material, in which basal and apical pompons could reproduce asexually by monosporulation.

No apical pompons occurred in the *Halymenia* cultures grown from carpospores produced by the Banyuls material.

Sori of "gemmae", as occurring on old *Halymenia* plants in cultures of Rovinj material, were not observed on old *Halymenia* plants in cultures of Banyuls material (3.1.1.3.).

3.1.2.1. Regenerative and reproductive capacity of basal pompons and apical *Halymenia* fragments

Morphological evidence suggested that asexual reproduction by monospores formed on the surface of the *Halymenia* thallus was much more important than asexual reproduction by monospores produced by basal pompons. To test this suggestion the following comparative cultures in 40 ml deep petri dishes containing 30 ml culture fluid were set up:

3 fragments of one basal pompon were transferred each into one of three petri dishes;

3 apical fragments of *Halymenia* plants were transferred each into one of three petri dishes.

In the course of the following two months it appeared that:

1. basal pompons as well as apical fragments of *Halymenia* plants were able to reproduce by monospores;
2. in both cases the monospores developed into *Acrochaetium*-like pompons

that were able to reproduce by monospores and to differentiate, in a limited number, into new *Halymenia* plants (*figs. 17–21*).

3. monosporic reproduction by the apical fragments of *Halymenia* plants was initially much more vigorous than by basal pompons.

After two months the three fragments of a basal pompon had produced a total of 34 pompons (diam. 2–3 mm) three of which were bearing young *Halymenia* plants. After two months the three apical fragments of *Halymenia* plants had produced a total of about 190 pompons (diam. $\frac{1}{2}$–2 mm) four of which were bearing young *Halymenia* plants.

After eight months the three fragments of a basal pompon had produced about 200 young pompons (1–3 mm diameter), 106 of which were bearing young *Halymenia* plants (0.1–2.5 cm high). Some of these young *Halymenia* plants were covered by warty monosporangial sori.

After eight months the three apical fragments of *Halymenia* plants had produced about 250 pompons (diam. 1–2 mm), 30 of which were bearing young *Halymenia* plants (0.3–1.5 cm high). The original apical *Halymenia* fragments had hardly grown at all but were covered by monosporangial sori.

It appeared that all young Halymenia plants derived from monospores produced by the apical Halymenia fragments were young gametophytes. These young plants were covered by pores sunk in depressions in the surface of the thallus (*figs. 22, 23*). Each pore opened on a gonimocarp initial as described by BERTHOLD (1884) for *Halymenia floresia* (*figs. 24, 25*). A detailed study of these initials was not possible with the limited material available. However, the following particulars could be observed. The gonimocarp initial is a flask-like structure ("flaschenförmiges Organ", see BERTHOLD 1884, p. 11) opening through a pore sunk in the surface of the thallus. The wall of this flask consists of a number of curved and branched enveloping filaments ("Hüllfäden") that arise from (one or more?) cortex cells. According to Berthold this flask-like structure contains either an auxiliary cell or a carpogonium (see *fig. 25* of a transverse section of a gonimocarp initial, probably showing the carpogonium).

According to Berthold the gonimocarp initial of *Halymenia* resembles very much that of *Grateloupia filicina*. In the latter species the enveloping filaments of a gonimocarp initial arise from an inner cortex cell as one fertile branching system bearing either the auxiliary cell or the carpogonium (*cf.* KYLIN 1930; 1956, p. 215). However, for *Halymenia* no detailed investigations on the structure of the gonimocarp initial are known to us; Berthold does not give much more than a rather rough picture of this structure (see also J. G. AGARDH 1879, t. 5, fig. 7).

Post-fertilization stages were not observed. According to Berthold many branching connecting filaments grow from a fertilized carpogonium to the auxiliary cells which, after contact with the connecting filaments, cut off the gonimoblast.

None of the young *Halymenia* plants derived from monospores produced by the three fragments of one original basal pompon were bearing gonimocarp initials.

The basal pompons grown from monospores produced by the apical *Halymenia* fragments were mostly very compact. In general, pompons of Banyuls material were much more compact than those of Rovinj material. Often the monospores produced by apical *Halymenia* fragments of Banyuls material had grown into basal lenticular multilayered discs with a distinct peripheral radial structure (*figs. 27-31*). Such basal discs and basal pompons were interconnected by a series of morphological intermediates, each partly a disc and partly a pompon.

Initials of *Halymenia* plants started each from a filament arising from a basal disc or a basal pompon. A *Halymenia* plant started as a terminal botryoid compact cluster of laterals arising from such a filament (*figs. 32, 33*). Therefore, ontogenetically *Halymenia floresia* plants appeared to be uniaxial, and only secondarily multiaxial.

3.1.2.2. Attempts to induce formation of tetrasporangia

The *Halymenia* plants grown from carpospores could possibly be tetrasporophytes. However, although they were regularly searched for tetrasporangia these were never observed.

In an attempt to induce the formation of tetrasporangia 5 *Halymenia* plants derived from carpospores of Banyuls material were transferred to 12 °C short day conditions. No tetrasporangia, however, were formed in a period of 7 months after transfer. Dissemination of monospores produced by the sori on the *Halymenia* thallus took place and these monospores developed into numerous young pompons, from none of which, however, developed new *Halymenia* plants.

The effect of aeration (under 17 °C long day conditions) was also tested on two *Halymenia* plants derived from carpospores. No tetrasporangia were induced in a period of 6 months, but monosporulation was much enhanced; the bottoms and sides of the 250 ml Erlenmeyer bottles were covered with thousands of new pompons.

3.2. Vegetative isolates of Halymenia gametophytes

Vegetative *Halymenia* isolates were obtained only from cortex filaments of gametophytes collected at Rovinj. After one month the isolates (a number of 10) had grown into *Acrochaetium*-like pompons 0.3–1 mm in diameter (*fig. 34*). In the original material it could be observed that *Acrochaetium*-like filaments arose from cortex-cells. These *Acrochaetium*-like pompons reproduced intensively by monosporulation, and young *Acrochaetium*-like generations were already profusely developing.

Since under 17 °C long day conditions plants of this *Acrochaetium* phase did not differentiate into *Halymenia* plants (not even in a period of more than one year!), it was subjected to the standard experiment to investigate combined influence of day-length and temperature on morphogenesis and reproduction.

The results of this experiment were negative; none of the sets of conditions (4 °C long day; 4 °C short day; 12 °C long day; 12 °C short day; 17 °C long day)

Fig. 1. Filamentous *Acrochaetium*-like phase grown from carpospores isolated from Rovinj material. One initial of the *Halymenia* phase (arrow).

Figs. 2–4. Successive growth stages of the same basal pompon and *Halymenia* plants arising from it (5 months, 6 months, and 7 months old, respectively) (Rovinj material).

Fig. 5. Longitudinal section of young *Halymenia* plant, showing the compact cortex and the very loose medulla. Young hyphae, arising from the inner cortex layers, are pictured (arrow) (Rovinj material).

Fig. 6. Subcortical layer of young stellate cells, viewed from the interior of a young *Halymenia* plant (Rovinj material).

Fig. 7. Surface view of compact outer cortex of young *Halymenia* plant (Rovinj material) Note the dense chromatophores.

Fig. 8. Detail of young hypha in medulla of young *Halymenia* plant (Rovinj material). Note the reduced, strap-shaped to reticulate chromatophores.

Fig. 9. Apical pompon (Rovinj material). Swollen cells in basal region of pompon.

Figs. 10, 11. Apical pompon (Rovinj material). Peripheral, more cylindrical cells.

Fig. 12. Gemma on old cultured *Halymenia* plant (Rovinj material). A gemma consists of a clump of spherical cells formed terminally on a long-celled filament growing through the cortex to the surface of the thallus.

Figs. 13–15. Transverse sections of monosporangial sori on *Halymenia* plants grown from carpospores isolated from Banyuls material.

Fig. 16. *Halymenia* plant (Banyuls material) grown from isolated carpospore. Surface completely covered by monosporangia. Note the characteristically contorted and warty surface.

Figs. 17–21. Filamentous phase grown from monospores produced by monosporangial *Halymenia* plants. Note the emptied monosporangia (arrow a) and a monospore (arrow b). Apical as well as intercalary cells can function as monosporangia.

Figs. 22, 23. *Halymenia* plant grown from a monospore produced by a monosporangial *Halymenia* plant (Banyuls material). Note the numerous pores sunk in depressions in the surface of the thallus. Each pore opens on a gonimocarp initial.

Figs. 24, 25. Transverse sections of depressions in the surface of the *Halymenia* plant pictured in *figs. 22* and *23*. Below the bottom of each depression a gonimocarp initial (Berthold's "flaschenförmiges Organ"). In *fig. 24* only a surface view of the enveloping filaments ("Hüllfäden") is pictured; in *fig. 25* a transverse section of the "flaschenförmiges Organ" is given, in which probably the carpogonium (arrow a) is pictured and a necrotic cell (arrow b) on the bottom of the depression.

Fig. 26. Gonimocarp initial viewed from the interior of a gametophytic *Halymenia* plant. The enveloping filaments are clearly distinguishable. Note the characteristic stellate cells of the subcortical tissue-layer.

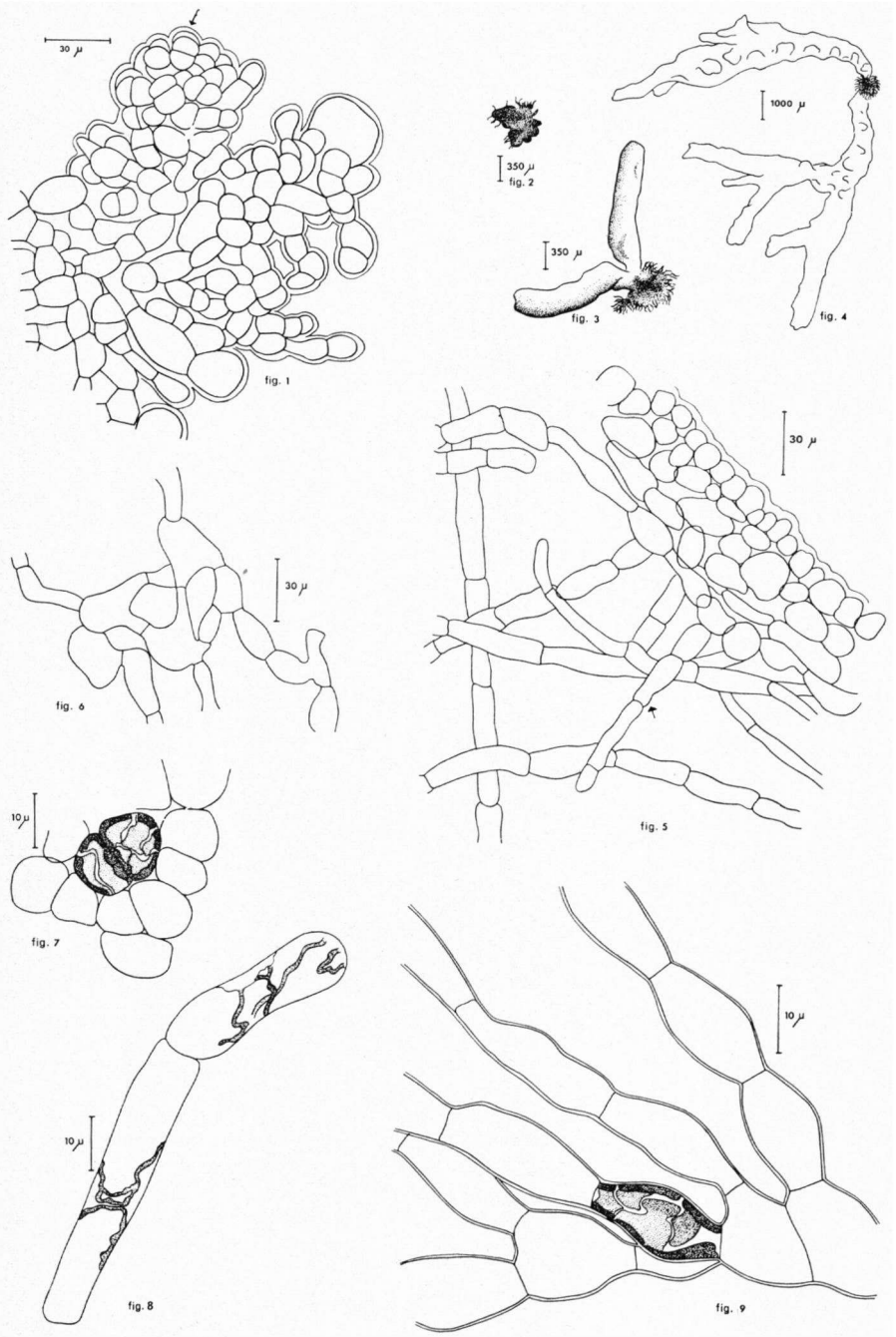
Fig. 27. Basal multilayered disc grown from a monospore produced by a monosporangial *Halymenia* plant. Numerous *Halymenia* initials (e.g. arrows) arising from the surface of the basal disc (Banyuls material).

Fig. 28. Margin of basal disc as pictured in *fig. 27* (surface view). Note the distinct radial structure. Particularly older cells are densely filled with floridean starch.

Figs. 29–31. Transverse sections of a multilayered disc as pictured in *fig. 27* (upper side, lower, side, and margin, respectively).

Fig. 32. *Halymenia* initial arising from basal disc (Banyuls material).

Fig. 33. *Halymenia* initial arising from filamentous stage (basal pompon) (Banyuls material).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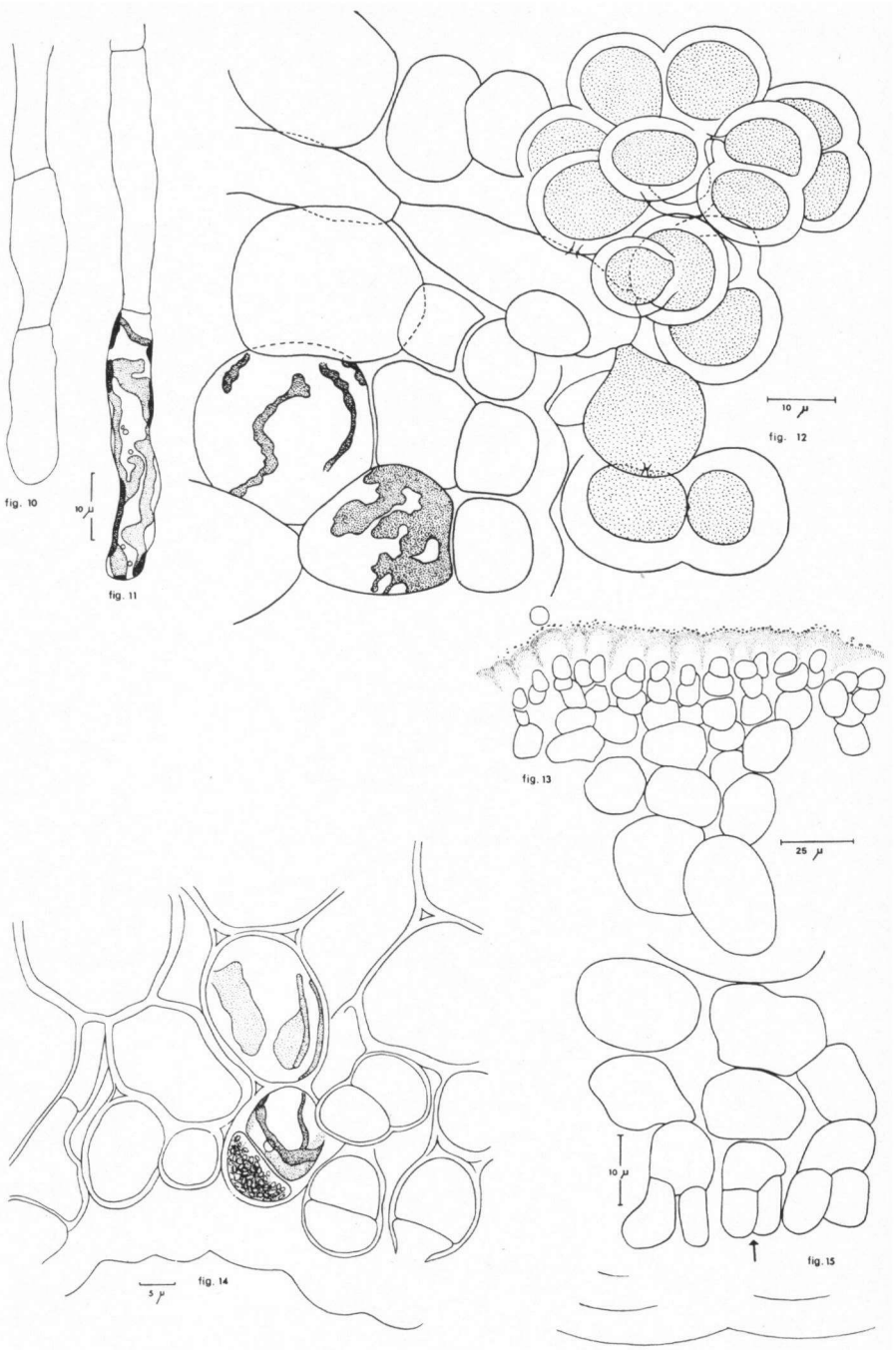
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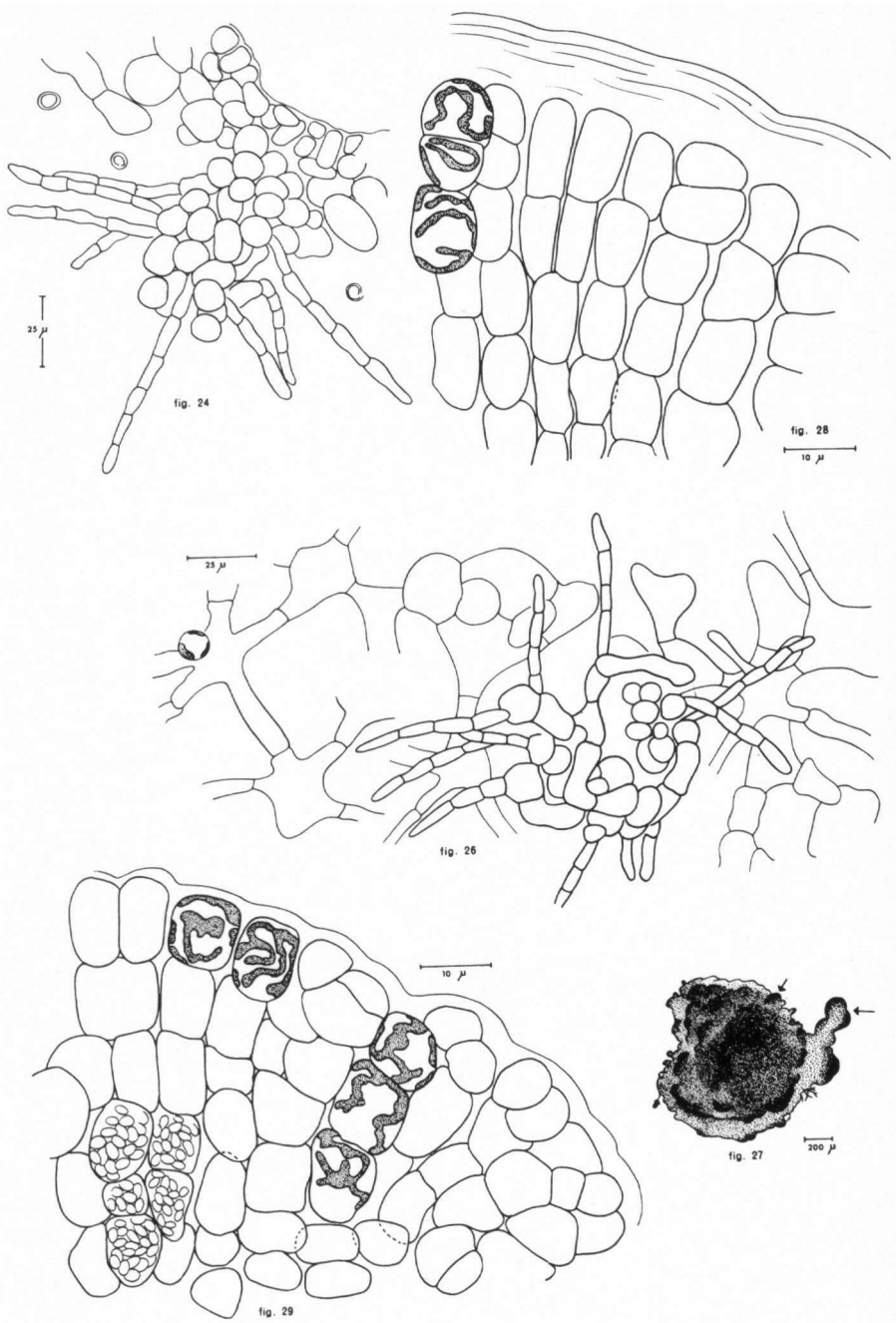
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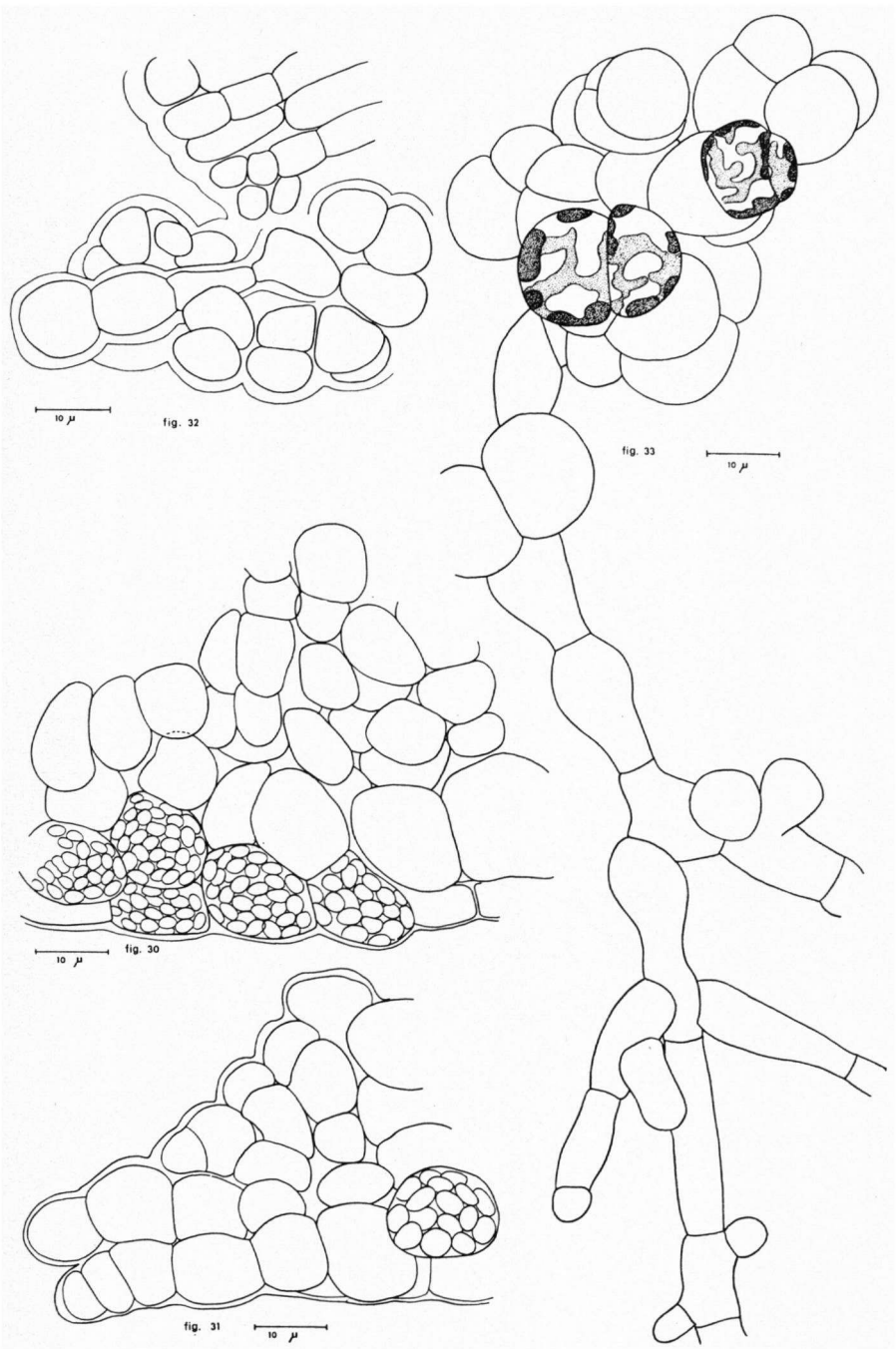
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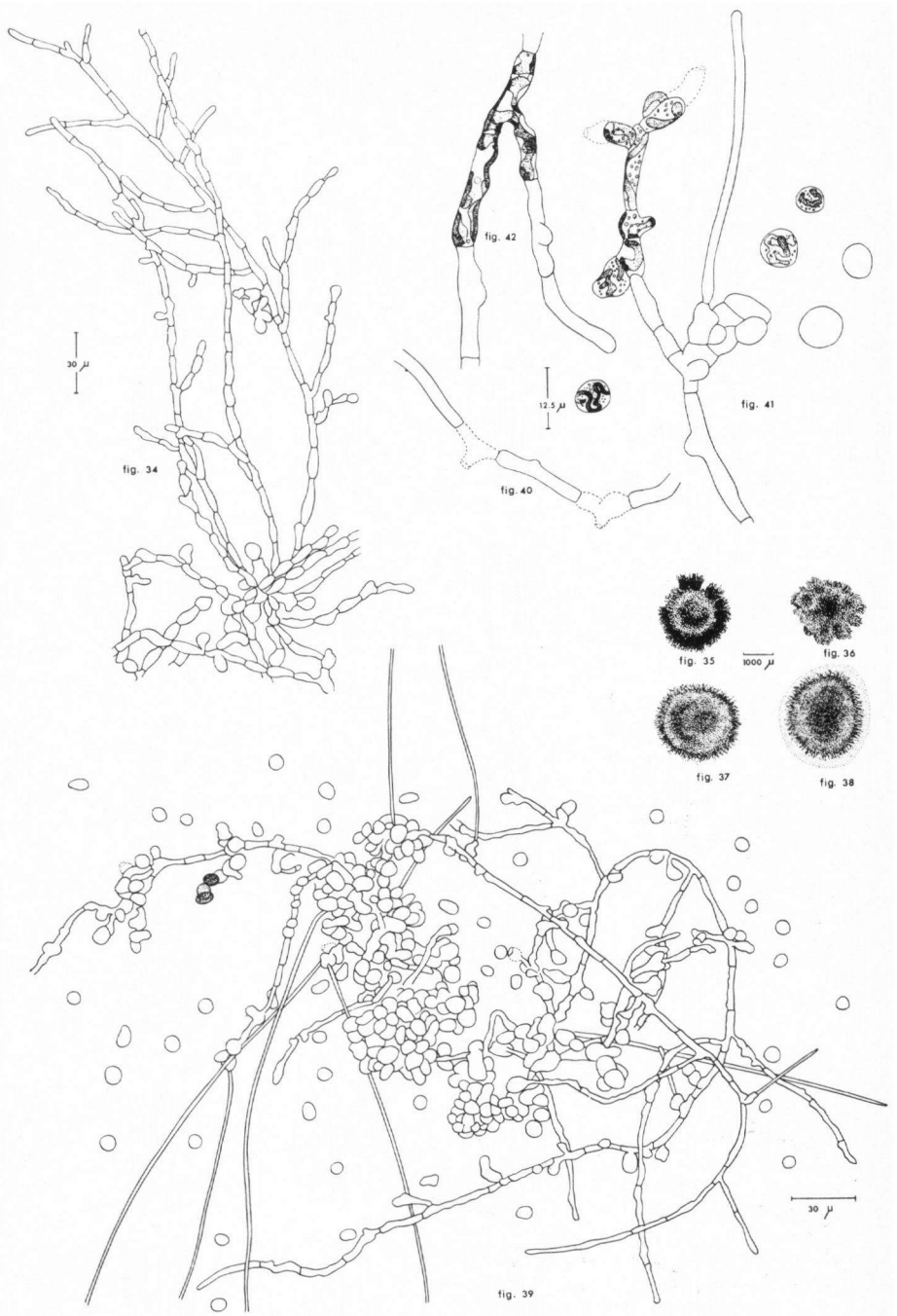
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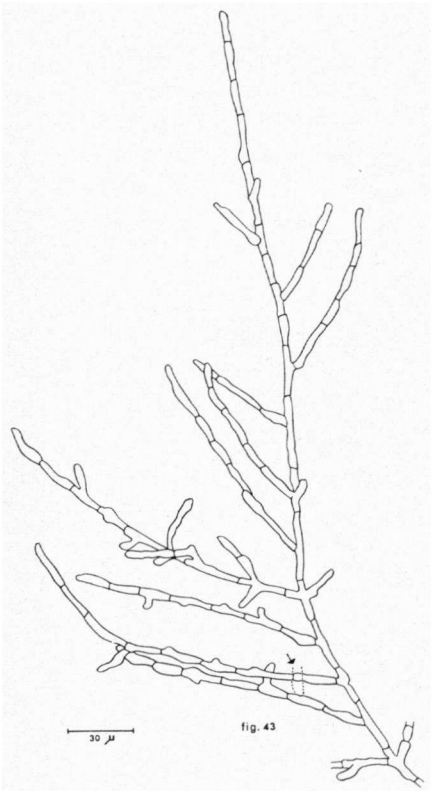

Fig. 34. *Acrochaetium*-like pompon grown from a vegetative isolate of a *Halymenia* gametophyte (Rovinj material). Note the short-celled basal filaments which attach the pompon to the substrate.

Figs. 35–38. *Acrochaetium*-like pompons grown, via monospores, from vegetative isolates of *Halymenia* gametophytes (Rovinj material). *Figs. 35-37* the type with relatively poor monosporulation. *Figs. 35, 36* older pompons surrounded by a ring of young pompons. *Fig. 38* the type with monospores forming a "halo".

Figs. 39–41. Details of an *Acrochaetium*-like pompon grown from a vegetative isolate of a *Halymenia* gametophyte (Rovinj material). The type characterized by extremely intensive monosporulation. Note the numerous monospores, which move by jerking amoeboid movements. Note, in *figs. 40* and *41*, the emptied monosporangia.

Figs. 42, 43. Details of an *Acrochaetium*-like pompon grown from a vegetative isolate of a *Halymenia* gametophyte (Rovinj material). Type with relatively poor monosporulation. Note emptied monosporangium in *fig. 43* (arrow).

Figs. 44, 45. Ca 11 months old *Halymenia* plants grown from carpospores (Rovinj material).

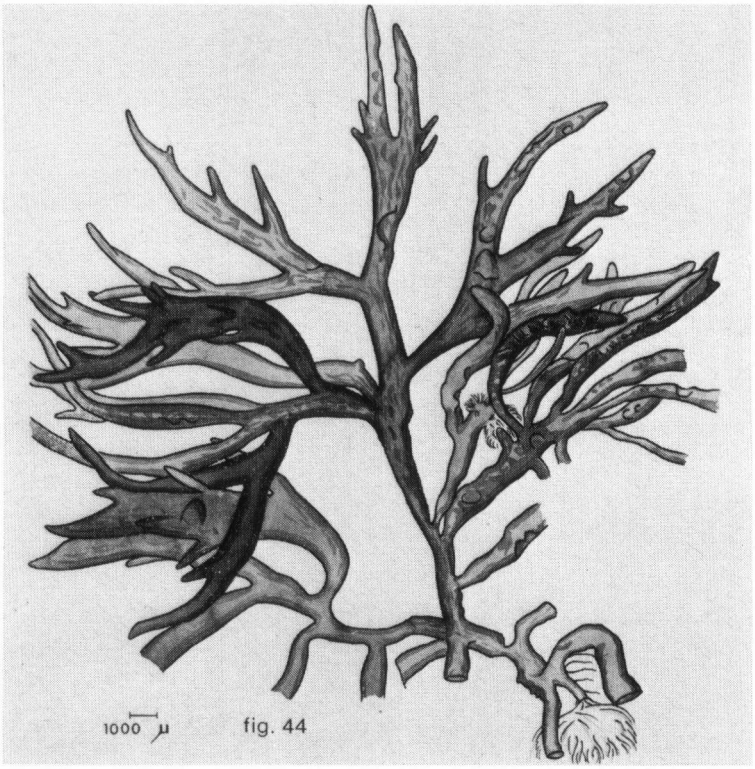

fig. 44

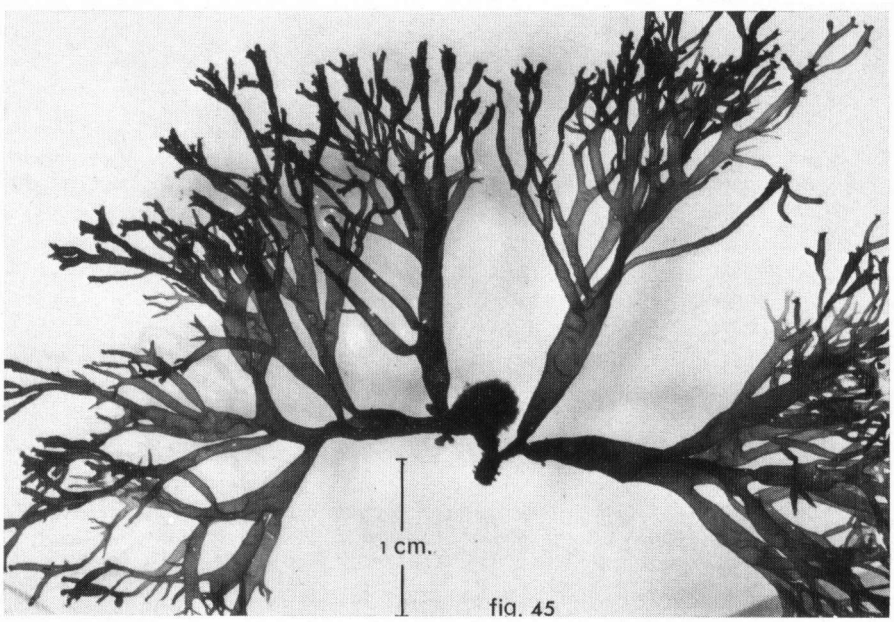

fig. 45

or changes of conditions induced the *Acrochaetium* phase to develop into new *Halymenia* plants.

All plants kept at 4 °C died within a few weeks.

The 12 °C and 17 °C cultures showed only differences in growth and reproduction, the 17 °C cultures showing a more rapid growth and more intensive monosporulation than the 12 °C ones.

However, another interesting aspect became apparent in the course of this experiment. It appeared namely that in the five clonal cultures used for the experiment at least three constant types could be distinguished.

1. The type characterized by extremely intensive monosporulation. Very young plants already formed monosporangia, and whole plants could be transformed into monosporangia as a result of which "plaques" of almost pure monospores could be formed (*figs. 39–41*). Within two months the bottoms of petri dishes containing this type were covered by a felty layer of filaments and monospores in which separate plants could hardly be distinguished.
2. The type characterized by intensive sporulation and by monospores forming a "halo" around the parent-pompon. The monospores crept away by amoeboid movements up to a short distance from the parent pompon. In this way they formed a "halo" around the parent pompon (*fig. 38*). In this type separate plants could be distinguished for much longer than in type 1.
3. The type characterized by much less intensive sporulation. Dissemination was much more restricted so that distinctly separate *Acrochaetium*-like pompons could be recognized for many months. Older pompons were often surrounded by a ring of young pompons sprouting from the central pompon (*figs. 35–37, 42, 43*).

Although several *Halymenia* gametophytes were used for isolating cultures it is not known whether the different clonal types were isolated from different plants.

4. CONCLUSIONS AND DISCUSSION

Halymenia floresia is a tropical to subtropical (sublittoral) species which is known from the Mediterranean, the Canaries, the Caribbean, and the coast of North Carolina (FUNK 1927, 1955; FELDMANN 1942; TAYLOR 1960; WILLIAMS 1951).

Isomorphic gametophytes and tetrasporophytes are reported in the literature, so that a heteromorphic life-history could hardly be expected for this species.

In the Mediterranean both gametophytes and tetrasporophytes have been collected only in spring and summer, and consequently both phases can there be considered summer-annuals. Observations in other parts of its geographic area are too scattered to permit generalizations about the time of the year at which *Halymenia* would be most likely to occur.

With regards to the Mediterranean *Halymenia floresia* there remained the problem in which form this species could hibernate.

In our investigations the life-history of *Halymenia floresia* from Rovinj

differed in several respects from the life-history of *Halymenia floresia* from Banyuls.

As we succeeded in culturing the life-history of the Banyuls *Halymenia* from gametophyte, via sporophyte, to gametophyte, we shall firstly summarise the particulars of this life-history.

Carpospores grew into filamentous, *Acrochaetium*-like pompons, which could give rise to new *Halymenia* plants, but which were also capable of reproducing themselves by monospores (*fig. 1*).

In all cases only a limited number of all *Acrochaetium*-like plants derived from monosporic disseminations of this phase developed *Halymenia* plants.

The *Halymenia* plants derived from carpospores were sporophytes. However, no tetrasporangia were produced by these sporophytes, but monosporangia grouped in dense sori on the surface of the thallus (*figs. 13–15*). Sporophytic *Halymenia* plants had a characteristic warty surface, and the fertile plants had a characteristically contorted habit (*fig. 16*).

Monospores produced by the *Halymenia* sporophyte grew into *Acrochaetium*-like pompons (*figs. 17–21*) or multilayered discs (*figs. 27–31*) or morphologically intermediate basal structures, from some of which young gametophytic *Halymenia* plants differentiated (*figs. 22–26, 32, 33*).

Temperature and daylength did not seem to have a marked influence on this life-history, which was cultured entirely under 17 °C long day conditions. However, sporophytes kept at 12 °C short day conditions produced new *Acrochaetium*-like plants none of which were capable of developing new *Halymenia* plants. This observation suggests that in winter, during which in the Mediterranean temperature and daylength roughly correspond to the 12 °C short day conditions in our experiments, the basal filamentous or disciform plantlets are not capable of developing *Halymenia* plants. Full-grown *Halymenia* plants, however, did not die under 12 °C short day conditions but even showed some growth and branching. The fact that during winter no new *Halymenia* plants can be initiated could account for a gradual decline of the summer *Halymenia* population and a complete retreat into the *Acrochaetium* or disc phase during winter.

Notwithstanding a regular search for tetrasporangia we did not succeed in finding them on the presumed sporophytic phase. According to literature data (KYLIN 1956; FUNK 1955; BØRGESEN 1915–1920) cruciate tetrasporangia lie embedded in the outer cortex layer of *Halymenia floresia* and other *Halymenia* species. Monospores produced by the sporophytic phase in our cultures of Banyuls material grew into gametophytes and thus presumably are functionally similar to tetraspores. The discrepancy between the reference, in the literature, to tetrasporangia in *Halymenia* and our observations could have two causes: firstly it is conceivable that the few observations on tetrasporangia actually refer to monosporangium mother cells cutting off monospores (some of our pictures indeed suggest cruciate tetrasporangia; see *figs. 13–15*); secondly it is possible that some *Halymenia floresia* populations have tetrasporangia-bearing sporophytes and other *Halymenia floresia* populations monosporangia-

bearing sporophytes. Our observations that monospores produced by the sporophytic *Halymenia* phase can grow into gametophytes suggest that meiosis takes place during formation of these monospores. However, other possibilities should not be excluded, e.g., that meiosis takes place at the moment a sporophytic *Halymenia* plant differentiates from a basal pompon, or a gametophytic *Halymenia* plant differentiates from a basal pompon or disc, in the way a *Lemanea* gametophyte differentiates from its basal system (MAGNE 1967). Anyhow, karyological investigations will be required to further elucidate the life-history of *Halymenia floresia.*

Halymenia floresia cultures of Rovinj material differed from cultures of Banyuls material in several respects.

Carpospores also grew into filamentous *Acrochaetium*-like pompons, which could give rise to new *Halymenia* plants (*figs. 2–4, 44, 45*), but which were also capable of reproducing themselves by monospores. Here also only a limited number of all *Acrochaetium*-like plants derived from monosporic dissemination of the *Acrochaetium* phase developed *Halymenia* plants. The *Acrochaetium*-like plants of the Rovinj cultures had a much less compact structure and could reach larger diameters than those of the Banyuls cultures (about 1.5 cm *versus* 0.3 cm).

The *Halymenia* plants derived from carpospores of Rovinj material neither produced tetrasporangia, nor monosporangia. Therefore they differed from comparable *Halymenia* plants of the Banyuls cultures in lacking monosporangial sori, and in never showing the characteristic habit of plants covered by monosporangial sori (compare *fig. 16* of a full-grown *Halymenia* grown from a Banyuls carpospore with *fig. 44* of a full-grown *Halymenia* grown from a Rovinj carpospore).

However, *Halymenia* plants grown from carpospores of Rovinj material had two additional means of vegetative propagation lacking in such plants of Banyuls material.

Full-grown *Halymenia* plants of Rovinj material became necrotic at their tips. Islands of living cells in the necrotic apical tissues could proliferate and give rise to *Acrochaetium*-like pompons termed apical pompons (*figs. 44, 9–11*). Apical pompons produced monospores that gave rise to new *Acrochaetium*-like pompons from some of which new *Halymenia* plants developed. Our apical pompons very much resembled the endophytic rhodophycea "*Endorhodophycea halymeniae*" described by FUNK (1955, p. 87, 161). This endophyte develops in degenerating *Halymenia* tissues of autumn plants.

A second means of vegetative propagation by full-grown *Halymenia* plants of Rovinj material was provided by the sori of gemmae on the surface of the thalli (*figs. 12, 46*).

Attempts to induce the formation of tetrasporangia by transfer to 12 °C short day conditions, by application of the standard experiment to investigate the combined influence of daylength and temperature, and by aeration were not successful, so that we did not succeed in completing the life-history of Rovinj-*Halymenia* in culture.

The observation that under 12 °C short day conditions the *Acrochaetium* plants could not develop into *Halymenia* plants suggests that during winter – which season in the Mediterranean roughly corresponds with our 12 °C short day conditions – *Acrochaetium* plants are not able to differentiate into new *Halymenia* plants. In this respect *Halymenia floresia* from Rovinj and from Banyuls resemble each other. It seems, therefore, very likely that *Halymenia floresia* hibernates as an *Acrochaetium*-like phase or as a disciform phase. This *Acrochaetium* phase (or the disciform phase of Banyuls material) most probably is a perennial, whereas both the sporophytic and gametophytic *Halymenia* phases are summer annuals. The sporophytic *Acrochaetium* phase at least is capable of a continuous asexual reproduction by monospores and is highly independent of both *Halymenia* phases. It is a matter of definition whether to call the life-history of *Halymenia floresia* isomorphic or heteromorphic. If the *Acrochaetium* phase is considered a juvenile stage of the *Halymenia* plant capable of independent reproduction, then the life-history of *Halymenia floresia* can be considered isomorphic. If, on the other hand, the *Acrochaetium* phase is considered an independent phase in the life-history of *Halymenia floresia*, from some individuals of which plants of the *Halymenia* phase can arise, the life-history can be considered heteromorphic. A comparable terminological difficulty applies to the life-history of *Lemanea*; are the diploid basal filamentous phase and the haploid erect *Lemanea* phase sprouting from the diploid phase to be considered one generation or two generations?

It would be interesting to know whether, in tropical regions, *Halymenia floresia* has a seasonal appearance or not. In the Mediterranean this species reaches its northernmost boundary and there in winter it retreats into its *Acrochaetium* phase (or disciform phase). In tropical waters with only slightly

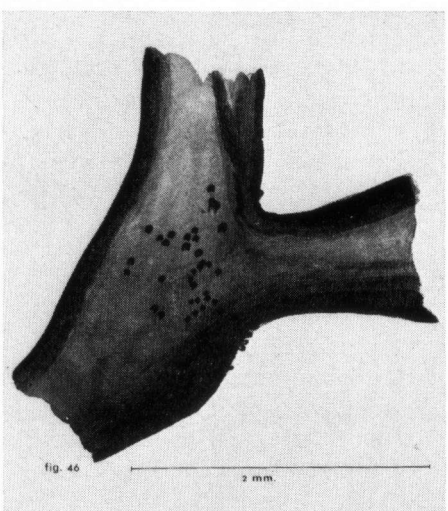

Fig. 46. Detail of an old *Halymenia* plant grown from a carpospore (Rovinj material), showing a sorus of gemmae.

varying temperatures and light conditions throughout the year it seems likely that *Halymenia* plants could differentiate from the *Acrochaetium* phase the whole year round.

Vegetative isolates from *Halymenia* gametophytes (Rovinj material) gave rise to *Acrochaetium*-like plants which were very similar to the *Acrochaetium* phase grown from carpospores and its monosporic offspring (*figs. 34–43*). Stock cultures of the original gametophytic *Halymenia* plants contained similar *Acrochaetium* plants after several weeks. Inspection of the original material showed that the *Acrochaetium*-like plants originated as proliferations from inner cortical cells. This *Acrochaetium* phase derived from cortical cells of the *Halymenia* gametophyte (Rovinj material) has apparently lost the capacity to redifferentiate into new *Halymenia* plants. Certainly cultures kept for almost two years never differentiated into *Halymenia* plants.

In fact these gametophytic *Acrochaetium* plants can be compared to the "apical pompons" of *Halymenia* plants grown from carpospores; the latter apical pompons however, are capable of redifferentiation into *Halymenia* plants. It seems, therefore, as if haploid apical pompons are not capable of redifferentiation into *Halymenia* plants, whereas diploid apical pompons are.

These gametophytic apical pompons of *Halymenia* can possibly be conceived as consisting of cells that have lost their totipotency, or, in other words, in which that part of the genetic information necessary for the morphogenesis of the adult *Halymenia* thallus is irreversibly blocked.

Our observations suggest that *Halymenia floresia* is capable of continuously producing, as a by-product of its life-history, *Acrochaetium*-like algae that are unable to return to this life-history. Since these *Acrochaetium*-like algae have their own means of reproduction, they can lead an independent life. At least three different clonal types (differing in growth form and intensity of monosporulation) could even be distinguished.

The above suggested mechanism of speciation in *Halymenia floresia* resembles the suggested hypothesis of the origin of algal species by the separation, through apomixis, of the two phases of a dimorphic life-history (DIXON 1963).

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