# A STUDY OF THE MARINE ALGAL EPIPHYTE
# Placophora binderi (J.Agardh) J.Agardh
# (CERAMIALES : RHODOPHYCOPHYTA)

by

Diana Hendy Hartley

Submitted in Partial Fulfilment of the
Requirements for the Degree of
Master of Science
in the Department of Plant Sciences
Rhodes University
Grahamstown

January 1986

# CONTENTS

# PREFACE

The experimental work described in this thesis was carried out in the department of Plant Sciences, Rhodes University, Grahamstown under the supervision of Professor S.C.Seagrief.

The study represents original work by the author and has not been submitted in any other form to any other university, except for two papers:

1) delivered at the Phycological Society of Southern Africa's (PSSA) 2nd annual conference in January 1984 entitled "Observations on the development and release of spermatia in Placophora binderi (J.Ag.) J.Ag." and published as an abstract in the PSSA'S newsletter number 15 September 1985.

2) delivered at the Electron Microscopy Society of Southern Africa's (EMSSA) 23rd annual conference in December 1984, entitled "Ultrastructure of carposporogenesis and tetrasporogenesis in the marine red alga Placophora binderi" and published as an abstract in the EMSSA'S proceedings, volume, 14 1984.

Where use has been made of the techniques or research of others it has been duly acknowledged.

## ACKNOWLEDGMENTS

Thanks are due to:

The C.S.I.R. for financial assistance.

Professor S.C.Seagrief for supervising the project, reading through the rough draft and providing helpful comments.

Mr. R.H.M.Cross of the electron microscopy unit (Rhodes University) for invaluable help and advice with transmission electron microscopy (TEM), scanning electron microscopy (SEM) and especially Cryo-Scanning electron microscopy.

Mr. A.H.Hartley of the electron microscopy unit (Rhodes University) for all the help, patience and advice with TEM, SEM and for printing the photographs which appear in this thesis.

Mr. R.S.Williams and Mr. J.Narsai for technical assistance with chemicals, apparatus and equipment.

Professor M.H.Hommersand and Mrs. F.Hommersand for their help, advice and for instruction in the technique of light microscopy optical sectioning.

Mr. A.Carter for all the informal discussion and for providing transport for numerous trips to the coast.

Mr. P.Gennrich-de Lisle for instruction in the operation of the word processor.

Mr. H.A.A.McKenzie, Mr. M.O.Sha, Mr. E.DeJager and Mrs. A.E.Hancocks of the Computer Science Centre for the help and guidance with operating the CPT 8100 word processor.

Mr. W.O.West of the Geography department for supplying the co-ordinates of the study sites.

Mr. M.Hanley for his time and effort in preparing bromide copies of the photographs and printing the copies of this thesis.

Ms. H.J.Kew for advice with the layout and format of this thesis.

Mr. J.E.Hodgkiss and Mrs. M.K.Wright for proofreading rough drafts of the final copy.

My parents for everything.

My husband Alex for his encouragement and help, without which this thesis would never have been completed! He also deserves heartfelt thanks for reading through the first drafts and giving many helpful comments of constructive disagreement.

## LIST OF FIGURES

# ABSTRACT

Placophora binderi can be described as an "obligate epiphyte" as it does not respond well to any culture conditions and is found growing only on other algae in the natural environment. This habit may have arisen as a response to the best available substrate in a harsh environment (Harlin 1971; Moss 1982). Any nutrient transfer which may occur between Placophora binderi and its basiphyte, usually various species of Codium, is probably by diffusion as rhizoids do not penetrate the basiphyte cells but simply lie between the Codium utricles providing better anchorage.

A triphasic life history exists with isomorphic gametophyte, carposporophyte and tetrasporophyte generations. The male and female gametophytes are dioecious.

This study confirms Scagel's (1953) observations for the development of the juvenile, mature and reproductive thallus. The juvenile develops as an erect polysiphonous thallus which produces a prostrate lobe as an adventitious branch from the basal segments. This prostrate lobe develops into the dorsiventrally flattened mature thallus. Reproductive structures are produced on erect branches which are initiated at the mature thallus margins. The gametophyte develops on evanescent trichoblasts produced on erect reproductive branches while the tetrasporophyte develops within

these erect branches. The female gametophyte has a four-celled carpogonial branch with an auxiliary cell forming after fertilisation from the supporting cell.

At the electron microscope level several vesicle types were seen in the reproductive organs. In the male, spermatial vesicles are produced which probably aid in release of the spermatia (Kugrens 1980). These are also visible under the light microscope. In carposporogenesis and tetrasporogenesis, three vesicle types are produced. Striated vesicles appear for a short while during the early stages and probably function as protein stores. Fibrillar vesicles are large and visible under the light microscope. These probably act as carbohydrate storage organelles (Triemer and Vasconcelos 1979; Kugrens and West 1973c; Tripodi 1971). Cored vesicles appear late in sporogenesis and probably aid in adhesion once the spores have settled (Chamberlain and Evans 1973; Wetherbee 1978).

Carpospores follow the "serial release" type pattern observed in Polysiphonia (Boney 1978). Tetraspores are released singly via a rupture in the tetrasporangial wall as in Ceramium rubrum (Chamberlain and Evans 1973). Both carpospores and tetraspores germinate in the typical bi-polar Ceramium-type pattern described by Dixon (1973).

# CHAPTER 1

# INTRODUCTION

## 1.1 CERAMIALES : RHODOMELACEAE

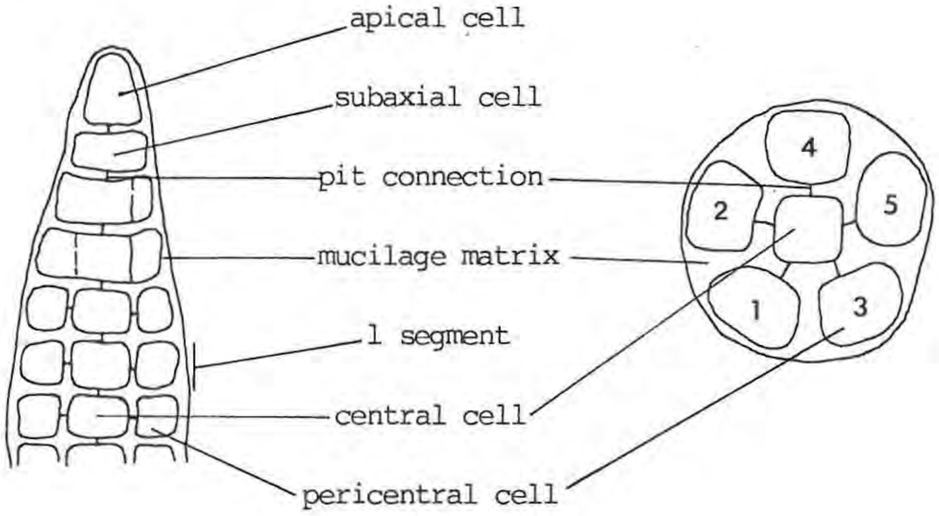
*Placophora binderi* is classified by Scagel (1953) and Seagrief (1980) as follows:

Division: RHODOPHYCOPHYTA
Class: Rhodophyceae
Sub Class: Florideophycideae
Order: Ceramiales
Family: Rhodomelaceae

Ceramiales is the largest order of "red algae" in terms of numbers of genera and throughout this order the female reproductive system is uniform. A vegetative cell within the thallus becomes fertile and is called the supporting cell from which a four-celled carpogonial branch and an auxiliary cell (after fertilisation has occurred) are always produced. The carpogonium and auxiliary cell are close spatially, usually within the same branch system, thus the order is said to be procarpial (Bold and Wynne 1978).

Life histories are usually diplohaplontic and isomorphic with dioecious male and female gametophytes. It is usually described as a "*Polysiphonia*-type life history", which is typically triphasic with tetrahedrally arranged tetraspores (Bold and Wynne 1978; Brauner 1979; Dixon 1973; Whittick 1981; Wollaston 1974).

Rhodomelaceae is the largest family of red algae with over one hundred genera which are located mainly in the southern hemisphere. Thalli are either filamentous or pseudoparenchymatous. In the latter the filaments are polysiphonous. An apical cell cuts off central cells (axial cells) proximally; these elongate and cut off pericentral cells laterally (Fig.1a). The pericentral cells are the same length as the central cells and as many as twenty four may be cut off per central cell. Each central cell with its pericentral cells is referred to as a segment (Scagel 1953).



a) Longitudinal section

b) Cross section

<u>Fig.1</u>: Polysiphonous filament construction. Numbers indicate sequence in which cells are cut off.

In the Rhodomelaceae a basic number of five pericentral cells are usually cut off and in an alternate sequence such that the last formed is opposite the first (Fig.1b).

Reproductive organs are usually borne on trichoblasts which are exogenous determinate branches of the vegetative thallus, arising from the subaxial cell before pericentral cells are cut off. They are either simple or branched and may become polysiphonous (Scagel 1953).

The gametophytes are usually dioecious. In the female gametophyte a pericentral cell of the suprabasal segment of the trichoblast becomes fertile and cuts off, in succession, a lateral sterile cell, a four-celled carpogonial branch and a second sterile cell. This pericentral cell is the supporting cell and cuts off the auxiliary cell after fertilisation. Direct fusion occurs between the fertilised carpogonium and the auxiliary cell, which then cuts off a gonimoblast initial from which gonimoblast filaments develop and these ultimately cut off carpospores. Cells of the carpogonial branch not used in gonimoblast production may fuse to form a fusion cell together with the sterile cells and this may have a nutritive function (Scagel 1953). The pericarp forms, before fertilisation, from the sterile pericentral cells on each side of the fertile pericentral cell. It develops as the carposporophyte matures and, at maturity, has an opening called the ostiole through which carpospores escape (Bold and Wynne 1978; Fritsch 1935; Hommersand 1963; Scagel 1953).

In the male gametophyte, each pericentral cell of a fertile trichoblast divides to produce a spermatangial mother cell which divides to form a number of spermatangia. One spermatium (the male gamete) is produced per spermatangium (Bold and Wynne 1978; Fritsch 1935; Hommersand 1963; Scagel 1953). The mature branch in which spermatangia are produced is called the spermatangial branch (Hommersand 1963).

In the tetrasporophyte, fertile polysiphonous branches (the tetrasporangial branches) produce tetrasporangia within each segment. A fertile pericentral cell cuts off two cover cells. It then divides to produce a tetrasporangium and a stalk cell. Tetrasporangia divide in a tetrahedral pattern (Fritsch 1935; Hommersand 1963; Scagel 1953).

These reproductive patterns are uniform throughout the family even though vegetative thalli are markedly diverse in structure. All genera are basically polysiphonous, with thalli showing simple filamentous, bilateral, radial or dorsiventral symmetry (Bold and Wynne 1978; Scagel 1953). Dorsiventral symmetry occurs by a longitudinal division giving rise to nonidentical halves. The Rhodomelaceae has been subdivided into a number of subfamilies depending on which symmetry thalli show (Scagel 1953).

## 1.2 PARASITISM AND EPIPHYTISM

Many genera within the Rhodomelaceae are parasitic or epiphytic (Bold and Wynne). Evans, Callow and Callow (1978) and Harlin (1971) define epiphytes as those organisms which grow on plants without actively drawing food or water from the living tissues. Linskens (1963) refers to the plant on which epiphytes grow as the basiphyte rather than the "host" as the latter term implies a dependent relationship by the epiphyte. This term is used in this thesis for the same reasons.

A number of species have been described as parasites, based largely on Setchell's (1918) definition of red algal parasites, namely: species which penetrate and form pit connections with the host, have reduced thalli and reduced pigmentation. However, recent experiments have shown that these criteria do not apply only to parasites but to some epiphytes as well. A more recent definition of parasitic algae includes all organisms which exist in intimate association with another living organism, deriving essential material for their existence with no return benefits (Evans, Callow and Callow 1978; Goff 1976). Two types of parasitic red algae are recognised: adelphoparasites, which are those species closely related to their hosts usually within the same family, and alloparasites which are those species not related below order level.

Several theories on the origin of parasitic algae have been proposed and were reviewed by Fan in 1961. The earliest theory is that of Setchell (1918), who claimed that mutant spores may have germinated and matured on parent thalli to give rise to the adelphoparasites. A more popular proposal is that of Sturch (supported by Fan (1961)) who believes that they developed from epiphytes. His reasons for this are:

(1) A large number of forms exist which are intermediate between the parasitic and epiphytic habits.

(2) The rhizoids of many epiphytes penetrate the surface of the "host" for better anchorage and are thus partially endophytic. Later a more dependent relationship could develop by these rhizoids taking up substances from the "host" tissue.

(3) Red algae have the unique ability to form connections with adjacent cells, particularly if the cells are from related species.

(4) Epiphytic species are often associated with a definite "host" species or a group of "host" species eg. Smithora naiadum on the seagrasses Zostera and Phyllospadix (Harlin 1971; 1973a).

Recently a number of experiments have indicated that species formerly described as parasites or obligate epiphytes have no real dependence on the basiphyte for nutrient uptake. Smithora naiadum, an obligate epiphyte on seagrass "hosts" (Harlin 1971) and the parasitic Janczewskia morimotoi (Court 1980) both grow and germinate on artificial substrates and show little or no transport of radiolabelled compounds between the basiphytes and the epiphyte/parasite. This indicates that the basiphytes are not required for substrate selection or thallus maturation. However, in both cases life histories were not completed on artificial substrate either *in situ* or under laboratory conditions, which indicates that the basiphyte may provide some biochemical factor, hormone or vitamin which stimulates thalli to reproductive maturity (Harlin 1971; 1973a). In another system, *Polysiphonia lanosa*, epiphytic on *Ascophyllum nodosum*, is capable of manufacturing all its own metabolites but is found only on this host. Thus, a hormone or vitamin may be required for early germination or the host surface may merely provide a suitable substrate for the settling spores (Harlin and Craigie 1975).

Transport of radiolabelled compounds such as carbon, nitrogen and phosphorus has been found to occur between basiphyte and epiphyte and vice versa in a number of species eg. between *Caulerpa* and its range of epiphytes, from *Codium* to *Polysiphonia* (Linskens 1963) and between *Zostera* and its epiphytes (McRoy and Goering 1974). However this does not necessarily imply active transport and parasitism (Citharel 1972). In many cases it is more likely to be a leakage of substances from one thallus and subsequent uptake by the other from the water immediately surrounding the algae (Harlin 1971;1973b; McRoy and Goering 1974). The close proximity of epiphyte and basiphyte would allow this type of passive transfer to occur (Fan 1961; Harlin 1971).

In well established parasitic species such as Holmsella pachyderma and Odonthalia flocossa, which are colourless, with endophytic cells establishing pit connections with host cells, direct nutrient transfer between host and parasite cells is likely (Evans, Callow and Callow 1973; Goff 1979a and b). In most relationships, however, it would appear that the primary role of the "host" is to provide a substrate and secondarily to provide biochemical factors via leakage or other indirect transfer processes. Rhizoids, although embedded in "host" tissue, would serve mainly as anchorage devices and if they provide the site for nutrient uptake it is likely to be a result of their proximity to "host" tissue.

Terms which imply any degree of dependence by one species of alga on another should be avoided until more detailed experiments can be done and more information is available on the biology of these relationships (Dixon 1973; Evans, Callow and Callow 1978; Fritsch 1935).

## 1.3 AIMS OF THE STUDY

There are two major aims to this study:

1) To determine the structure of Placophora binderi at the light microscope and electron microscope levels, the latter of which has not been done for this species.

2) To determine to what degree Placophora binderi is dependent on its basiphyte species.

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1 COLLECTING AND SAMPLING

Material was collected mainly at Kenton-on-Sea (33°41'30"S 26°40'30"E) and at Port Alfred (33°26'80"S 26°53'30"E) on the Eastern Cape coast, South Africa. One collection was made at Scottburgh (30°17'S 30°45'30"E) on the Natal coast, South Africa. Random samples of a number of "green", "brown" and "red" algae were taken to check for numbers and types of basiphyte species. During these collections other possible substrates were checked for *Placophora binderi*, eg. rock surfaces, sand, sedentary animals, flotsam and jetsam.

## 2.2 GROSS MORPHOLOGY

### Fresh or Formalin Preserved Specimens:

Specimens of *Placophora binderi* were removed from the basiphyte thallus and vegetative, male, female and tetrasporic thalli dissected out. Fresh specimens were mounted directly onto clean glass slides in a drop of sea water, while preserved specimens were mounted in a drop of 4% formalin in sea water. Clean glass coverslips were applied and sealed around the edges with clear nail varnish to prevent evaporation. The fresh specimens tended to disintegrate within a few days while the preserved specimens could be maintained in perfect condition for up to one year if sealed properly and kept in the dark to prevent pigments from bleaching.

## Nuclear Staining:

Specimens preserved in 4% formalin in sea water were placed in 10% formalin in sea water in plastic petri dishes under constant light and allowed to bleach for 14 days to remove all colour from the thalli. Specimens were then stained for two hours with Whittman's (1965) Aceto-Iron-Haematoxylin Chloral Hydrate (see appendix), dehydrated in 100% alcohol vapour overnight and mounted in Piccolyte after replacing the alcohol with xylene. This method stains nuclei and pit connections very well leaving the cell wall and ground cytoplasm only faintly coloured. It also allows good optical sections to be obtained in order to observe layers of cells.

## Thick Sections:

As part of the procedure for transmission electron microscopy (described under section 2.5), thick sections were cut with an LKB 8800 ultramicrotome 111 and placed on a drop of clean distilled water on a glass slide. The water was evaporated off in an oven at 60°C. Sections were stained with toluidine blue (see appendix) for 15 seconds in the oven, washed with tap water, dehydrated in an alcohol series and mounted in Depex.

## 2.3 RHIZOID STRUCTURE AND PENETRATION INTO THE BASIPHYTE

Rhizoid structure and penetration were observed using light microscopy (section 2.1), electron microscopy (section 2.5) and dissection under a Zeiss dissecting microscope. Placophora binderi thalli were gently pulled away from basiphyte thalli and it was noted whether rhizoid tips were whole or snapped off. If they were snapped off, more careful dissection was undertaken to remove these from the basiphyte tissue and to see whether rhizoids penetrated basiphyte cells or if they lay between them. Basiphyte tissue included various Codium species, Zonaria subarticulata and a piece of brown algal thallus densely covered with epiphytes.

## 2.4 SPORE RELEASE

Mature cystocarps and tetrasporangia of Placophora binderi were removed and placed on clean glass slides in a drop of filtered sea water. Coverslips were applied and slightly raised by placing thin strips of dampened filter paper on opposite sides of the coverslip to prevent squashing of the material. These were then observed continuously under low light for up to 12 hours under an Olympus Vanox photomicroscope and a Zeiss Phase Contrast photomicroscope. As soon as spore release appeared to be under way photographs were taken at timed intervals.

## 2.5 CULTURE EXPERIMENTS

Specimens required for culture purposes were collected, shaken free of water and transported back to the laboratory in a cooler bag and dealt with immediately. Best results for culturing isolates of Placophora binderi were obtained by placing mature cystocarps and tetrasporangia on glass coverslips or filter paper in deep, opaque, plastic containers (15x15 cm) with filtered, sterilised sea water. These were left for 48 hours in the dark to allow for maximum spore release. Adult thalli were removed and the containers with released spores were placed on a laboratory bench in an air conditioned room where normal daylength times could be experienced. After one week, one drop of Erdschreiber's enriched sea water medium (McLachlan 1973; Provasoli, McLaughlin and Droop 1957) (see appendix) was added to each dish. The culture medium, which contained 1mg/l germanium dioxide to limit diatom growth, was changed after two weeks. Germination and sporeling development were recorded using an Olympus Vanox photomicroscope or a Zeiss Phase Contrast photomicroscope.

Field cultures were attempted on artificial substrates; polythene strips, perspex slides and sponge rubber cubes were tied to Codium thalli with fishing line. The chosen basiphyte thalli had heavy infestations of Placophora binderi on them to maximise the chance of germination on the artificial substrate. The experiment was checked fortnightly.

Cultures on basiphyte thalli were attempted. Spores were allowed to release onto relatively epiphyte free portions of Codium under the culture conditions as described above. In addition mature segments of Placophora binderi were placed on basiphyte thalli and left to see if these would regenerate.

## 2.6 ULTRASTRUCTURE

The material was used either immediately or, when this was not possible, was shaken dry, placed in plastic bags and kept in a dark cool place for not longer than 24 hours.

Placophora binderi was removed from the basiphytes, which were predominantly species of Codium, roughly torn into pieces approximately 3mm$^2$ and treated according to the technique required.

### Transmission Electron Microscopy (TEM):

Material fixed either immediately or no longer than 3 hours after collection gave the best results. Fixation in 5% glutaraldehyde in fresh sea water (see appendix) for at least 48 hours, with one change of fluid at 24 hours, proved adequate. Material was then washed for 15 minutes in each of 25%, 50% and 75% 0.1M pH 7.0 cacodylate buffer (see appendix) in filtered sea water mixtures and finally in two changes of 100% buffer for 10 minutes each. Secondary fixation with 1% cacodylate buffered osmium tetroxide (see appendix) for 4 hours was followed by two washes in buffer for 15 minutes each. Material was dehydrated in an alcohol series of 30%, 70%, 80%, 90% and 100% ethanol. Following immersion in two changes of propylene oxide for 15 minutes each, material was infiltrated with increasing concentrations of TAAB 812 resin (see appendix) for extended periods of time not less than 12 hours. Standard infiltration times resulted in inadequate resin penetration. Pieces of material were then transferred to Pelco flat embedding molds and polymerised at 60°C for approximately 30 hours. Resin blocks were trimmed and reorientated on old stubs as required and sectioned with a diamond knife on an LKB 8800 ultramicrotome III.

Sections were stained with uranyl acetate (see appendix) for 28 minutes followed by Reynold's (1963) lead citrate (see appendix) for 7 minutes. Stained sections were viewed with a JEOL JEM 100CX II transmission electron microscope.

The ultrastructure of newly released carpospores and tetraspores was observed by allowing spores to release overnight from excised adult portions onto 2.5cm Whatman's Glass microfibre GF/C filter paper in deep 5cm opaque plastic containers filled with twice filtered, autoclaved sea water. The entire disc of filter paper together with released spores was then subjected to the same procedure as above except that the graded series of buffer/sea water washes were omitted after glutaraldehyde fixation.

## Scanning Electron Microscopy (SEM):

Material was fixed and dehydrated in the same way as for TEM. It was infiltrated with amyl acetate in a transitional series of 25:75, 50:50, 75:25 amyl acetate:ethanol mixtures and finally in 100% amyl acetate for 15 minutes each. The material was then critical point dried with carbon dioxide and sputter coated with gold in a Polaron E 5100 sputter coater. A JEOL JSM U3 scanning electron microscope was used to view the specimens.

## Cryo-Scanning Electron Microscopy:

Only freshly collected material was used. Excess sea water was removed from exised portions of female and tetrasporic thalli with absorbent paper. The specimens were glued onto stubs with wallpaper glue. The entire stub was immersed in slushy nitrogen to allow freezing to occur and then transferred to a Hexland

Cryo-System attached to a JEOL JSM 840 scanning electron microscope. The specimens were fractured and allowed to etch to a desirable degree. Etched specimens were coated with gold and viewed with a JEOL JSM 840 scanning electron microscope.

# CHAPTER 3

# GROSS MORPHOLOGY

## 3.1 INTRODUCTION

### The Genus Placophora

Placophora was first described by J.Agardh in 1841 from South African specimens epiphytic on species of the green alga Codium (Scagel 1953). Seagrief (1984) has listed two species as occurring in South Africa: Placophora binderi (J.Agardh) J.Agardh and Placophora monocarpa (Montagne) Papenfuss 1965. In 1953 Scagel reviewed and described Placophora from South African material epiphytic on Codium a "green alga" as well as on Corallopsis a "red alga" and on Pyura stolonifera an invertebrate. It has also been recorded as being present on the red algae Gelidium versicolor, Gelidium amansii, Amphiroa bowerbankii and Amphiroa ephedraea; on the "green alga" Halimeda and on the "brown alga" Zonaria subarticulata (Scagel 1953; Seagrief 1967). It has also been found in Peru, Japan, South Australia and Tristan da Cunha and may be restricted to the southern hemisphere (Fritsch 1935; Scagel 1953).

Placophora has a dark red-brown colour and exists as a flattened thallus on the basiphyte species. The flattened thallus forms as a result of congenital fusion of a highly branched polysiphonous system (Fritsch 1935; Scagel 1953). The juvenile thallus consists of an erect polysiphonous axis 3mm in height, the basal segments of which give rise to the flattened prostrate thallus (Scagel 1953). The reproductive thallus and developmental stages as described by Scagel (1953) conform to the pattern found throughout the Rhodomelaceae (chapter 1).

## Spore Release and Germination Patterns in the "Red Algae"

Studies on the mechanism of spore release in the "red algae" indicate that two types of release occur with respect to carpospores. The "mass release" type (Boney 1978) occurs where all the spores are forcefully released from the cystocarp. Under laboratory conditions the spores of *Rhodymenia pertusa* were released by prodding cystocarps with a blunt glass needle. In nature the trigger for spore release would probably be changes in water pressure or movement as the tides change. The spores are carried away from the parent plant and eventually the spore mass breaks up. This type of spore release has been seen in *Champia*, *Gracilaria* and *Rhodymenia* (Boney 1978). The other type, "serial release", occurs where large spores pass singly through the ostiole of the cystocarp as in *Polysiphonia* (Boney 1978) and *Ceramium rubrum* (Chamberlain and Evans 1973). Carpospores leave the spore mass within the cystocarp as elongate structures and move towards the ostiole. Once through the ostiole the spore rounds up, with the pole which was last to leave the ostiole rounding up first. Spores rotate during release with contents in constant motion. A rigid wall is lacking, which aids the passage of the spore through the ostiole and the rounding up process.

Tetraspore release was also studied in Ceramium rubrum (Chamberlain and Evans 1973). Here spores were released either singly by rupture of the tetrasporangium and thallus wall or, more frequently, the entire tetrasporangium was released. Spore release from the tetrasporangium followed the same pattern in both cases. Spherical spores were released singly through an opening, usually by rupture, in the tetrasporangial wall. They were greatly constricted during their passage through the opening and on release the spores rounded up again. Spore contents were in constant motion as in carpospores. In Catanella caespitosa, a similar release pattern is described where a zonate tetraspore pattern occurs (Prud'homme et al 1983).

Once released, spores may be carried away by water currents and eventually sink and settle on a new substrate. Spore attachment to a new substrate is most probably achieved by the production of mucilage which would act as an adhesive (Boney 1981; Fritsch 1935). Carpospores and tetraspores of any given species usually germinate in the same way (Fritsch 1935). Dixon (1973) lists five types of germination pattern which occur in the "red algae":

1) Nemalion-type: A germ tube is produced into which spore contents pass and is cut off by a transverse wall. The germ tube divides to produce a short filament; a rhizoid forms from the lower end and the thallus from the upper half. This is usually found in the Nemalionales, eg. Cumagloia andersonni (South 1968) and Bonnemaisonia nootkana (Chihara 1965).

2) Naccaria- type: A number of divisions occur within the spore without spore enlargement. One or more protruberances form which act as apical cells and eventually produce filaments. This occurs in some Nemalionales, eg. Rhodochorton concrescens (West 1970), some Gigartinales, eg. Chondrus crispus (Chen and McLachlan 1972) and in the Cryptonemiales.

3) Gelidium-type: Similar to Nemalion-type in that a germ tube is produced except that here one pole forms a rhizoid immediately and the other divides to produce the vegetative thallus. This is found in all Gelidium species, Gelidiella acerosa (Chihara and Kamura 1963), some Cryptonemiales and some Gigartinales, eg. Dermocorynus montagnei (Guiry and Maggs 1982).

4) Dumontia-type: A massive cell mass is formed from which erect fronds develop. Found in some Gigartinales, Cryptonemiales and Rhodymeniales.

5) Ceramium-type: Bipolar germination occurs where two opposite primordia are produced: one forms a rhizoid and the other the apical cell of the vegetative filament. This occurs exclusively in

the Ceramiales, eg. *Membranoptera multiramosa* (Waaland and Kemp 1972), *Janczewskia morimotoi* (Nonamura 1979) and *Crouania pleonospora* (Prince 1979).

## 3.2 AIMS

This part of the investigation set out to add to Scagel's (1953) information on the structure and development of the vegetative and reproductive thallus of *Placophora binderi*. Spore release was investigated to determine its mechanism. Random sampling was done to check for numbers of basiphyte species and to see if *Placophora binderi* is exclusively an epiphyte. Rhizoid structure and function were studied to determine whether or not they serve as anchorage devices only or if some other function also exists. Culture experiments were carried out to determine whether this epiphyte could survive with or without its usual basiphyte species and to see what type of germination pattern *Placophora binderi* has.

## 3.3 RESULTS

### Structure of the Vegetative Thallus

### a) The Juvenile Thallus

Very young and juvenile thalli of Placophora binderi were collected from "clean" areas on Codium.

Juveniles were usually observed at a fairly late stage of development having several rhizoids, an erect polysiphonous branch up to twenty segments long, and one to four small prostrate basal lobes (Figs. 2 and 3). The polysiphonous branch possesses an apical cell which cuts off segments below. The lower five segments have fewer pericentral cells than the main part of the branch which has a central cell and five to eight pericentral cells. The lobes are produced from the lower segments. Usually only one lobe develops from the erect juvenile branch which appears to be an adventitious branch from the pericentral cells (Fig.3).

The prostrate lobe becomes polysiphonous soon after formation and produces numerous branches in a radial fashion. The erect branch disintegrates by the time the prostrate thallus is 0.72mm long and 0.58mm wide at the widest part (Fig.4). The mature thallus continues to disintegrate at its origin as the thallus margins mature.

In a few instances areas where rejuvenation was occurring were observed. These were reminiscent of the newly formed prostrate lobes of juvenile thalli, growing out from the apical margin of the mature thallus (Fig.5).

### b) The Mature Thallus

The mature thallus is prostrate and deeply lobed. It has numerous congenitally fused polysiphonous branches, each branch containing a central cell and five pericentral cells. Three pericentral cells are present on the dorsal surface and two on the ventral surface (Fig.6). The central cells appear as "veins" on the thallus, each flanked by two of the three dorsal pericentral cells (Fig.7). The middle pericentral cell lies directly over the central cell and is thus not visible. The cells in mature regions of the thallus are usually more or less rectangular in shape while those near the apical margin are smaller and more square (Fig.9).

Branching occurs at the apical margin by an apical cell dividing obliquely to produce a secondary axis (Fig.6). The apical cell of the secondary axis divides periclinally until a branch is produced when it divides obliquely. This branching is regular. Two branches are produced to the right followed by two to the left, then two to the right again (Fig.8). At the thallus margin some areas branch extensively while others do not (Fig.7) resulting in the lobed character of the thallus. This branching pattern is just discernible in young adult thalli (Fig.4).

Pit connections are produced between every central cell and between the central cell and pericentral cells within a branch. Occasionally pit connections occur between pericentral cells within a branch or between branches (Fig.6). These are small structures and not always easily seen even under oil immersion.

Cells are uninucleate for approximately eight cells back from the margin, after which they become multinuclaeate (Fig.6) with up to five nuclei per cell. Multinucleate cells also tend to contain large central vacuoles with peripheral cytoplasm whereas marginal cells have an evenly distributed cytoplasm. The cytoplasm is a dark red-brown colour in unstained material.

Separate male, female and tetrasporic thalli are produced which are isomorphic in the vegetative state.

## Structure of the Reproductive Thallus

Reproductive thalli are recognisable by the production of clusters of erect branches at or near the thallus margins. These start by apical cells dividing obliquely, at right angles to the dorsiventral axis (Fig.9).

An apical cell develops which soon cuts off five pericentral cells to produce short, erect polysiphonous branches (Fig.10). In some cases these branch again to produce evanescent, colourless monosiphonous trichoblasts. The reproductive structures are borne on these erect branches or on the trichoblasts.

## a) The Development of the Carpogonial Branch and Cystocarp

On the female thallus up to four trichoblasts are produced near the apex of the erect branches (Fig.11). A carpogonial branch is produced on one of these trichoblasts, eventually resulting in one carposporophyte being produced per erect branch (Fig.12). Within a cluster of erect branches various stages of carposporophyte development can be found (Fig.12). The trichoblasts are monosiphonous except for the two basal segments which are polysiphonous. The carposporophyte develops from cells in the suprabasal segment while the basal segment merely supports the cystocarp (Fig.12). The monosiphonous segments tend to disappear as the carposporophyte matures.

In figure 13 a supporting cell is present and attached to it are the four-celled carpogonial branch with a trichogyne coming from the terminal cell, a two-celled and a four-celled sterile group. Two observations indicate that fertilisation has just occurred (Scagel 1953); the terminal cell of the carpogonial branch is well defined and all the cells attached to the supporting cell have an equal stain intensity. The pericentral cells have divided to produce a few cells of the bi-layered pericarp which surrounds the young carposporophyte cells. The monosiphonous segments of the trichoblast are still visible. The four-celled carpogonial branch and bi-layered pericarp are clearly shown in figure 14.

The supporting cell cuts off another cell, the generative auxiliary cell and the carpogonium extends a process towards this cell by which the fertilised nucleus is transferred. At this stage the carpogonial branch cells are still darkly stained but pit connection between them have disintegrated. The supporting cell and sterile cell groups are lightly stained with thickened pit connections. The pericarp is now definitely bi-layered (Fig.15).

Figure 16 shows a slightly later stage where the carpogonial branch cells are no longer evident. The central cell, pericentral cells, supporting cell, sterile groups, auxiliary cell and the gonimoblast initial are clearly visible. Very darkly stained spherical regions within the supporting and sterile groups are evident. A degree of fusion seems to have occurred. Cells have increased stain affinity and the pit connections are thickened. The pericarp has developed to the point where an ostiole has formed (Fig.16b). Young pericarp cells are uninucleate, becoming multinucleate later as in vegetative cell development (Fig.16b). Figure 17 shows a similar stage to that in figure 16 and in addition it shows that trichoblasts have disappeared and the basal segment from which the carposporophyte develops is clearly visible.

Once the gonimoblast initial has developed, the supporting cells and sterile cells fuse to form a fusion cell and the gonimoblast initial divides to produce gonimoblast filaments which cut off carpospores from their terminal ends. They contain a dense granular cytoplasm, central nucleus and one to three vesicular structures. The pericarp continues to develop as the carposporophyte forms producing a large oval to round cystocarp (110µm in diameter), made up of the bi-layered pericarp with an ostiole and the internal carposporophyte of gonimoblast filaments, carpospores and the fusion cell (Fig.18).

### b) The Development of the Spermatangial Branch

Three to four trichoblasts form at the apical end of reproductive branches, each of which becomes a spermatangium bearing branch (Figs. 19 and 20).

Trichoblasts become polysiphonous (Fig.19) with the two basal, the apical cell and up to three sub-apical segments remaining monosiphonous and sterile (Fig.20). The remaining segments usually produce five pericentral cells, each of which may become or produce spermatangial mother cells. The spermatangial mother cells cut off small spherical to ovoid spermatangia which mature acropetally (Fig.20).

Longitudinal sections show that spermatangial branches possess elongate central cells. Occasional pit connections can be seen between spermatangial mother cells and the central cells. The spermatangia appear as small elongate bodies 5.7µm long to 2.5µm wide with dense apical regions and highly vacuolar basal regions (Fig.21).

### c) The Development of the Tetrasporangial Branch

Trichoblasts were never seen on erect branches of tetrasporangial thalli (Fig.22). Tetrasporangia are produced singly in each of the segments and mature acropetally (Fig.22 and 23). They appear as extremely dark reddish brown spheres within the branches. In some oblique sections clear lines are visible which represent the cleavage furrows between two or more spores (Fig.23).

A fertile pericentral cell cuts off two cover cells (Fig.24). It then divides again to produce the tetrasporangium and the old pericentral cell becomes the stalk cell (Fig.25). The contents of the tetrasporangium then divide to produce four spores in a tetrahedral pattern. This is probably a meiotic division as complete division does not occur until near maturity and, in section, three nuclei are frequently visible together with three cleavage furrows. Several large vesicles are also visible within a dense cytoplasm (Fig.26).

## Spore Release

Carpospores were released singly from the cystocarp. A single spore breaks away from the spore mass within the cystocarp and moves through the cavity to the ostiole as an elongate structure, taking three to five seconds to traverse the cavity. Within one second the spore clears the ostiole as an elongate body with highly pigmented contents except for clear areas at the poles (Fig.27 a-d). The spore is squeezed slightly and rotates as it passes through the ostiole. During this process the entire cystocarp appears to "shiver" indicating an active, forceful process of spore release. Within one minute of release the spore rounds up into a spherical body, usually with the end which emerged last rounding up first giving the spore a pear shaped appearance, with the clear area remaining as a slight bump until the spore is completely rounded off (Fig.27 a-d). During the rounding up process the spore contents are in constant motion. Several spores may be released from one cystocarp within minutes of each other. No fixed rate of spore release could be determined.

Newly released tetraspores, identified by their contents being in motion and their proximity to an empty tetrasporangium within the branch, were seen as four separate rounded spheres very similar in appearance to newly released carpospores.

Spore release in the laboratory occurred at approximately the same time at which high tide would have occurred. This was noted the first time the release mechanism was observed. Subsequent observations were thus limited to a three hour period over which high tide was occurring.

## Culture Experiments

Cultures of spores on filter paper and glass microscope slides were equally successful. The glass slide substrate allowed for easier observation of early germination events. Carpospores and tetraspores showed identical germination patterns.

Spores were released within twelve hours of their being placed in culture chambers. Newly released spores were highly pigmented with a clear area at one pole and had diameters of 30µm (Fig.28). By three days colourless, uniseriate rhizoids of two to four cells had been produced which were approximately 100µm long and 15µm wide and usually arose as a colourless bump from the clear area (Fig.29). The opposite pole had divided to produce the first vegetative shoot of up to four cells which was approximately 35µm broad and 59µm long and highly pigmented. Both rhizoid and vegetative shoot cells arose as a result of simple transverse divisions (Fig.28 and 29). Both poles developed simultaneously. By ten days rhizoids had increased to approximately 147µm long and the vegetative shoot to 117µm long where the polysiphonous structure had also begun to form (Fig.30). A few spores showed malformation by developing lateral buds rather than vegetative shoots (Fig.31). Spores did not develop beyond that described at ten days and at fifty six days the experiment was stopped.

Addition of germanium dioxide to the cultures eliminated diatoms. Nutrient medium allowed spores to survive longer and germinate beyond the 3 cell stage. However, it also allowed invertebrate contaminants to flourish which obscured observation and in some cases resulted in spore death. Contaminants were a major problem and could be eliminated only by pipetting isolated spores to new culture chambers at regular intervals. This was a risky procedure due to the reluctance of spores to germinate under most culture conditions.

Spores which had degenerated due to contaminant interference or other factors could be seen by the presence of a "halo" of contaminants adhering to the thick mucilage sheath surrounding each spore.

The field culture experiments and attempts to culture Placophora binderi on its customary basiphyte were unsuccessful.

## Rhizoid Structure

The rhizoids of Placophora binderi are colourless and are produced from the pericentral cells on the ventral surface of the thallus. They are monosiphonous, septate and vary from unbranched to slightly branched on Codium basiphytes (Fig.32) to having basal ramifications on more solid basiphytes such as Zonaria. Rhizoids form a short distance back from the apical margin in clusters and at scattered intervals (Fig.33). On Zonaria basiphytes the rhizoids tend to be produced in bands corresponding to the "joints" seen on Zonaria thalli. Rhizoids also tend to be produced directly under areas where erect reproductive branches are formed.

Gentle pulling or dissection of mature and juvenile thalli of Placophora off the basiphytes allowed rhizoids to come away intact (Fig.32). On Codium, rhizoids of the epiphyte tend to penetrate a short distance between utricles while on Zonaria they tend to form cushions at the "joint" areas (Fig.36). The thallus of Placophora binderi usually lies approximately 64μm above the basiphyte thallus and not directly on it (Fig.34).

## Sampling

Placophora binderi was found at all the easily accessible places along the Eastern Cape coast from Port Elizabeth (35°57'S 25°36'E) to East London (33°02'S 27°55'E). It was found mainly on the erect Codium spp., eg. Codium duthieae Silva and Codium platylobium Areschoug and never on Codium lucasii Setchell which is a compact species and clings to rocky substrates. These species are found in tidal pools in the intertidal zone of the seashore. Occasionally Placophora binderi was seen on Zonaria subarticulata and on unrecognisable seaweeds which were heavily infested with other epiphytes. In the two major collection sites it was not recorded on any other species of alga, animal, flotsam or jetsam. On the Codium basiphytes mature Placophora binderi was only ever found on old portions of the thallus which were heavily infested with other species of epiphyte as well. Very young sporelings were found on "clean" areas and older portions of the basiphyte.

## 3.4 DISCUSSION

### Structure of the Vegetative and Reproductive Thallus

Early germination patterns and the structure of young thalli taken off the basiphyte agree with Scagel's (1953) account of juvenile development. The regions of rejuvenation appear to arise and develop in the same way as the prostrate lobes, producing new polysiphonous axes. Whether this mechanism originates by the division of the central cell or the apical cell is unclear as only well established areas of rejuvenation were observed.

The structure of the mature thallus and the development of the female, male and tetrasporophyte agrees with that described by Scagel (1953). The method of branching may provide a clue as to how the mature vegetative thallus originates. The original main axis of the prostrate lobe may begin branching fairly early after its formation, producing two secondary axes to the one side followed by two to the opposite side. Each of these branches again resulting in the radial branching pattern seen in very young adult thalli.

Once fertilisation has occurred and the carpogonial branch cells are no longer visible, the darkly stained spherical regions within the partially fused supporting cell/sterile cell group may be nuclei. These nuclei would belong to the supporting cell/sterile cell group and possibly the nuclei from the carpogonial branch. The latter may fuse with this complex and not merely disintegrate once fertilisation has occurred. If disintegration does occur then the extra nuclei may come from initial fusion with the inner pericarp cells. Eventually all the cells of the original carpogonial branch, sterile group and the supporting cell fuse together with some inner pericarp cells to form the fusion cell from which the gonimoblast filaments probably derive nutrition to produce carpospores (Scagel 1953).

## Spore Release

The mechanism of the release of carpospores follows that described by Boney (1967). The very clear area observed at the tip of the emerging spore which persists to rounding up is the area from which the rhizoid develops. This indicates that in Placophora binderi the change in shape of the emerging spore does affect subsequent polarity of the sporeling (Boney 1967).

Tetraspore release appears to follow that described by Chamberlain and Evans (1973) for Ceramium rubrum. They are probably released singly once the sporangial wall has ruptured. They round up and divide in the same way as carpospores.

Spores are not moved very far from the parent in culture, which may be due to lack of water movement or it may represent a natural occurrence which would account for the dense colonisations usually found on any one basiphyte thallus.

Spore release in the laboratory coincides with high tide. Ngan and Price (1983) make a similar observation in their study on tropical florideophycideae where maximum spore discharge occurred when populations on the shore would be submerged in sea water. This phenomenon may represent the method by which the trigger for spore release works. Pressure changes caused by changing tides or the movement of the blades in the water as tides shift may provide the necessary stimulation for spore release as in Rhodymenia pertusa (Boney 1978).

## Germination Pattern

Tetraspores and carpospores show the typical bi-polar Ceramium-type germination pattern (Dixon 1973). The malformed spores, also observed by Prud'homme et al (1983) but not commented on, may be the result of culture conditions or they may be naturally deformed and would probably die in the field.

The "halo" of contaminants is a commonly seen phenomenon of dead spores in culture (Boney 1981) and is attributed to the possible function of the mucilage as an antibiotic layer (Kugrens and West 1973a).

## The Epiphytic Nature of Placophora binderi

Many different methods exist for the culturing of algae eg.:

1) using only sterile seawater and changing it fortnightly

(Chihara 1965; Chihara and Kamura 1963; Edelstein 1970; Tveter and Mathieson 1976)

2) using enriched seawater and changing it regularly (Chen 1977; Chen, Edelstein and McLachlan 1974; Chen and McLachlan 1972; Edelstein and McLachlan 1971; Magruder 1977; Ogata, Matsui and Nakamura 1972; Stegenga 1978; Waaland and Kemp 1972; Whittick 1981; Whittick and West 1979). South (1968) and Swale and Belcher (1963) used agar as one of their culture substrates with as much success as other substrates. Placophora binderi spores also grew well on agar but disintegrated sooner than the the spores on glass slides or filter paper.

3) using enriched seawater, germanium dioxide and changing the medium regularly (Nonamura 1979; Polanshek and West 1975; South 1968). Suneson (1982) used the natural substrate of shells to get the cultures to reach maturity. His alga was the coralline Dermatolithon litorale and he proposes that it needed the extra calcium provided by the shells. West (1970) and Gordon-Mills and Womersley (1974) did not change the medium but thinned the sporelings when they were growing well. Germanium dioxide will not kill animals but will kill diatoms and is lethal to Rhodopyhcophyta if used in higher concentrations than 2ml/l. It should also not be used continually, only initially to rid the cultures of diatom contaminants (Markham and Hagmeier 1982).

4) using enriched seawater, germanium dioxide, Penicillin G and

changing the medium regularly (DeCew and West 1981; Guiry and Maggs 1982; Polanshek and West 1977; Shevlin and Polanshek 1978; West 1972; West, Polanshek and Shevlin 1978). Most of these authors also used microdissection to eliminate some contaminants. Penicillin G is used to kill bacteria and "blue-green" algae and as neither of these was a problem it was not used in these experiments.

Placophora binderi germinated only if released in sterile seawater and left there for one week, after which continued growth required the addition of enriched seawater. The medium was never changed as this caused sporelings to die. Diatom contaminants were eliminated with germanium dioxide. Invertebrate contaminants were a problem and could be eliminated only by microdissection or spore isolation both of which resulted in spore death. Guillard's (1973) advice was adhered to in that he states that it is important that the culture is growing well and all contaminants contained before microdissection or sporeling isolation is attempted.

The unsuccessful field culture experiments were probably due to a number of possible factors. Areas chosen for the experiment were subject to heavy wave action which probably resulted in the basiphyte being torn off due to extra weight and drag created by tying artificial substrates to it. Although carefully mapped, every experimental site had lost the experimental basiphytes a fortnight after installing them. A really strong anchorage device with attached artificial substrate pushed into the substrate on which *Codium* grows would probably prove more successful.

Attempts to germinate released spores or excised pieces of mature *Placophora binderi* thallus on whole *Codium* thalli or pieces were unsuccessful. This may be due to the reluctance of *Codium* to grow under culture conditions (personal experience and that of the technical staff of the department) and possibly the siphonous nature of the *Codium* thallus does not allow it to survive as cut pieces. Nonamura (1979) found that *Janczewskia morimotoi* spores would not reach maturity in culture unless its host, *Laurencia nipponica*, was present. Thus this epiphyte probably needs the host for some growth factor. Apt (1984) found that spores of the so-called parasite *Phaeocolax kajimurai* germinated to the two-celled stage only and that excised mature axes grew well with or without the host *Lobophora variegata*. Thus this species does not necessarily depend on the host for survival. It is found only on this host and no penetration of the host cells occurs, thus Apt (1984) calls it a host specific epiphyte.

Harlin (1971) believes that it is possible to force any epiphyte into a free living state by duplicating the exact physiological and environmental conditions for growth in the labrotary. *Placophora binderi* will probably grow in culture if the exact natural conditions are met. These would include approximating tidal pool conditions eg. variations in temperature, light and water movement as would be experienced with incoming and outgoing tides.

In this study *Placophora binderi* was found mainly on the erect *Codium* species. Scagel (1953) does not mention the frequency of sitings on other basiphytes merely that they were observed. This study seems to suggest that *Placophora binderi* uses *Codium* merely as a substrate which is easily penetrated. The fact that the firmer *Codium lucasii* does not support many epiphytes favours this suggestion.

Nutrient uptake by *Placophora binderi* would be due to simple diffusion by leakage from the old *Codium* tissue to the space between it and *Placophora* (Harlin 1971,1973b; McRoy and Goering 1974). The close association of rhizoids with *Codium* utricles would aid this process.

Rhizoids grow between the utricles of *Codium* and never appear to penetrate them. On basiphytes such as *Zonaria* where the thallus is firm the *Placophora* rhizoids form cushions on the surface. This change in structure of the rhizoid tip probably indicates penetrability or impenetrability of the basiphyte tissue (Scagel 1953).

Fig. 2: Juvenile thallus.

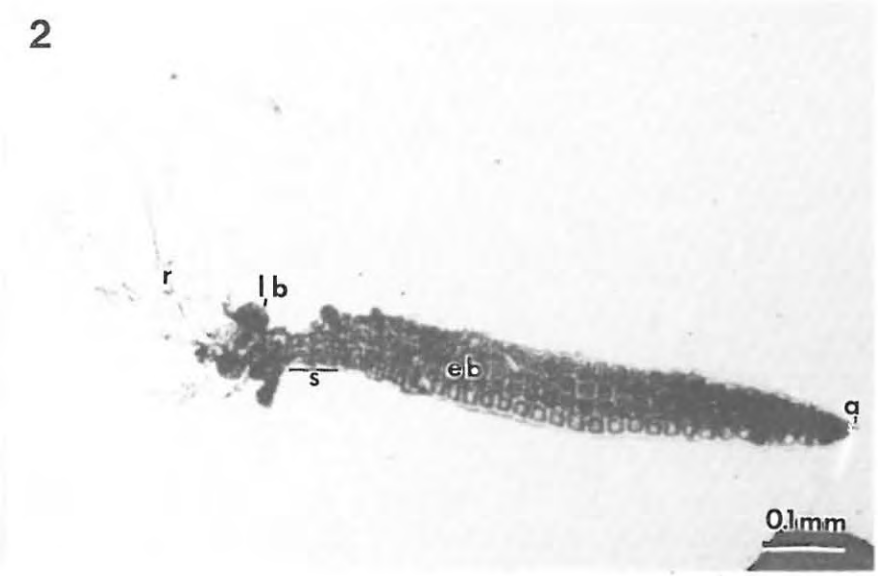
Fig. 3: Juvenile thallus.

Fig. 4: Young polysiphonous vegetative thallus where the erect branch has disintegrated.

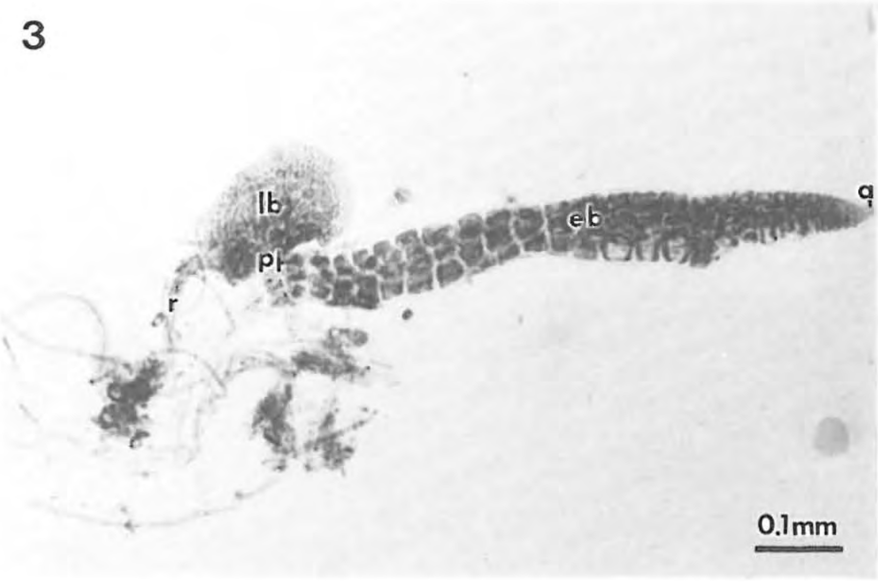
LEGEND:

a- apical cell
eb- erect polysiphonous branch
lb- prostrate basal lobe
o- thallus origin (where the erect branch disintegrates)
pl- pericentral cell from which the lobe is emerging
r- rhizoid
s- lower segments with fewer pericentral cells

2



3



4

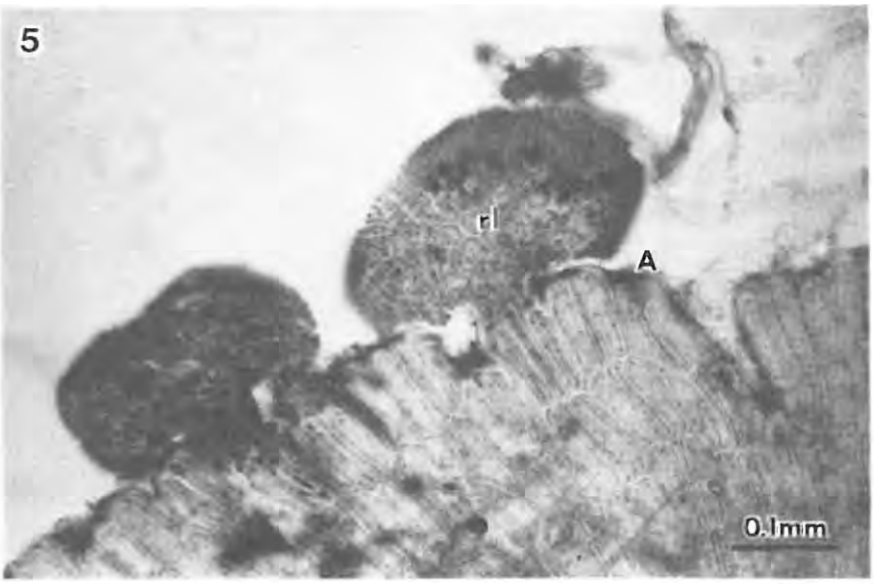
Fig. 5: Mature vegetative thallus with two rejuvenating lobes.

Fig. 6: Mature vegetative thallus. Drawn with the aid of a camera lucida.

LEGEND:

A- apical margin
a- apical cell
cc- central cell
mc- multinucleate cells
p- pit connection
pc- pericentral cell
rl- rejuvenating lobe
uc- uninucleate cells

5



6

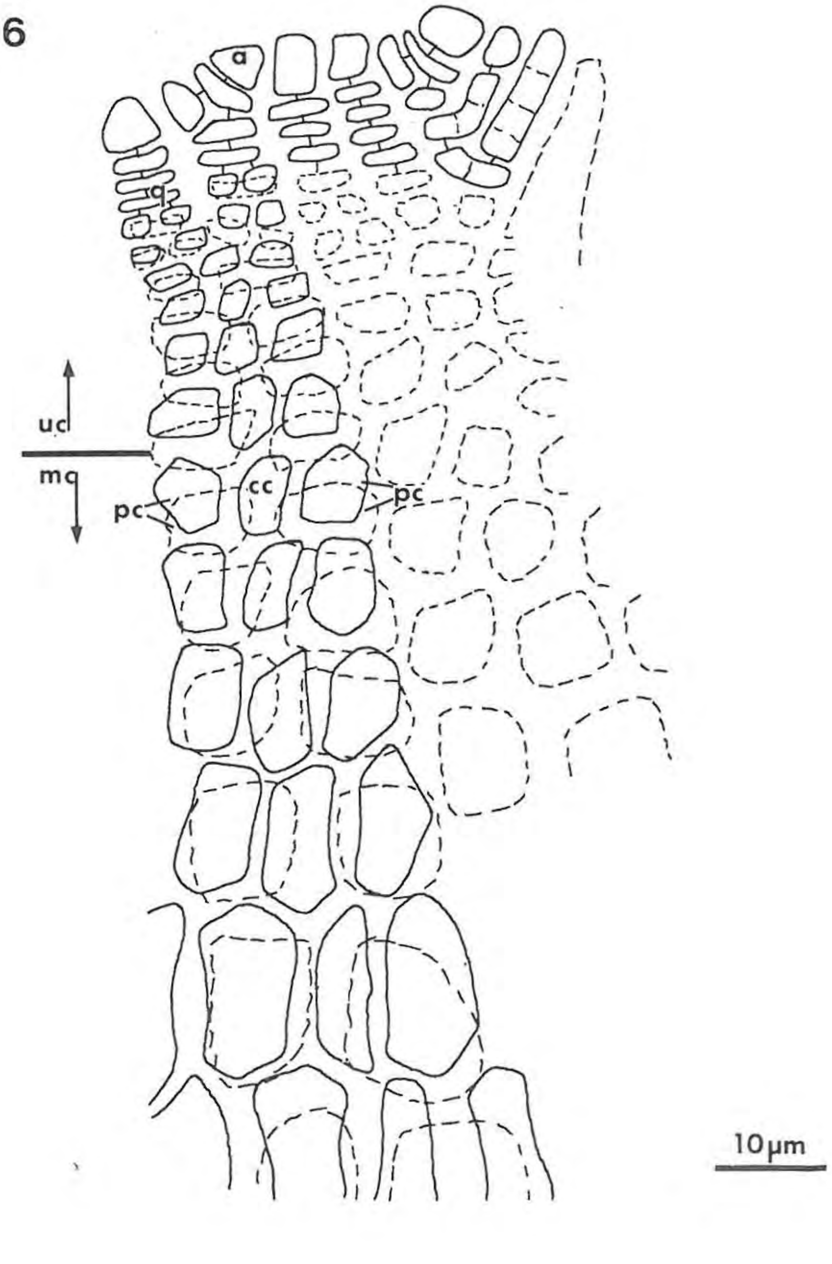


Fig. 7: Mature vegetative thallus.

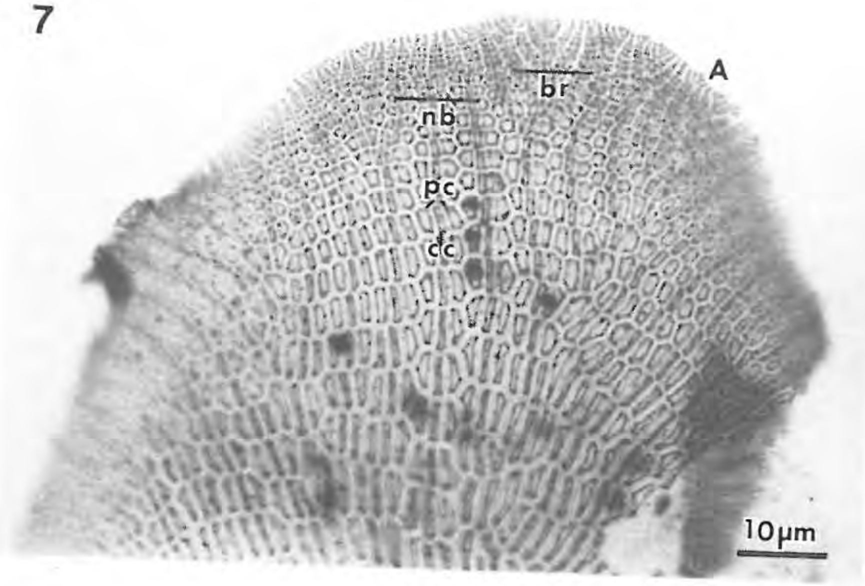
Fig. 8: Line diagram of the "veins" of central cells (main axes) to show the branching pattern of a mature vegetative thallus.

Fig. 9: Early stages in the development of the erect reproductive branches at the thallus margin. Drawn with the aid of a camera lucida.

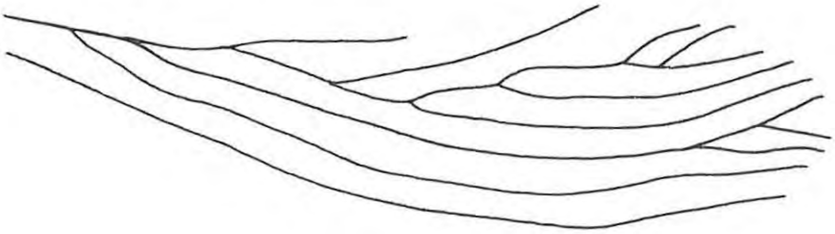
LEGEND:

A- apical margin
br-branching region
cc- central cell
mu- mucilage
nb- non-branching region
p- pit connection
pc- pericentral cell
u- upright branch

7



8



9

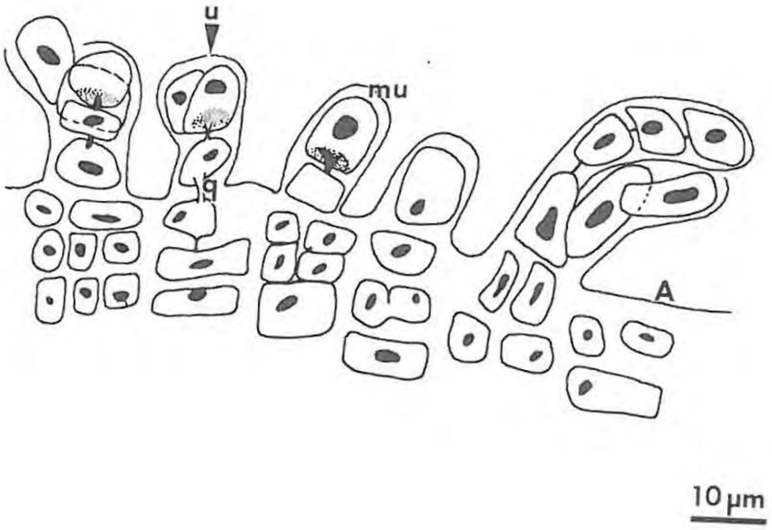


Fig. 10: Short, newly formed, erect polysiphonous reproductive branch. Drawn with the aid of a camara lucida.

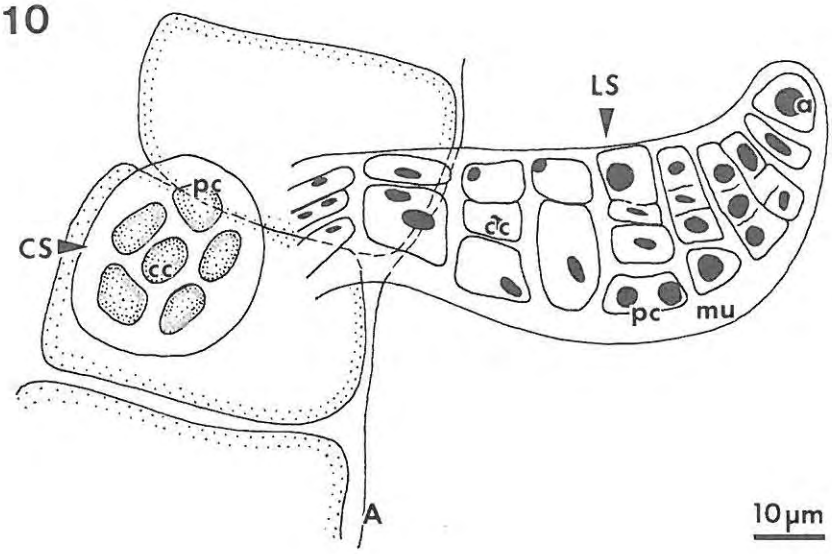
Fig. 11: Young female reproductive branches.

Fig. 12: Cluster of female reproductive branches with various stages of cystocarp development.

LEGEND:

A- apical margin
bs- basal segment
CS- cross section
c- carposporophyte
cc- central cell
cy- cystocarp
E- erect reproductive branch
LS- longitudinal section
mu- mucilage
pc- pericentral cell
T- trichoblast

10



11

12

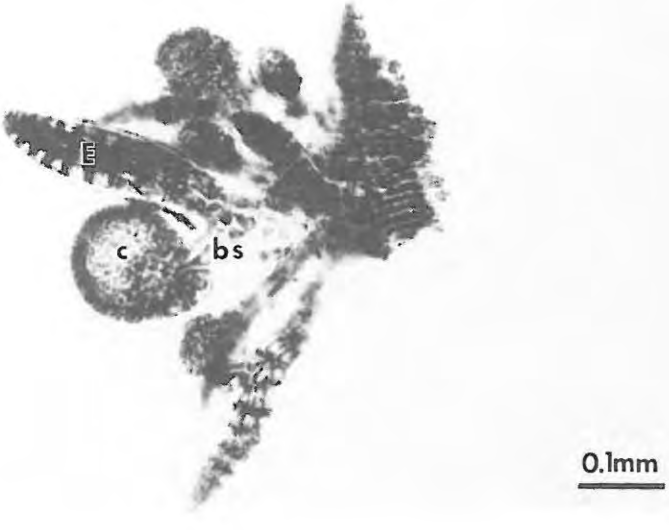


Fig. 13: Young post-fertilisation cystocarp.

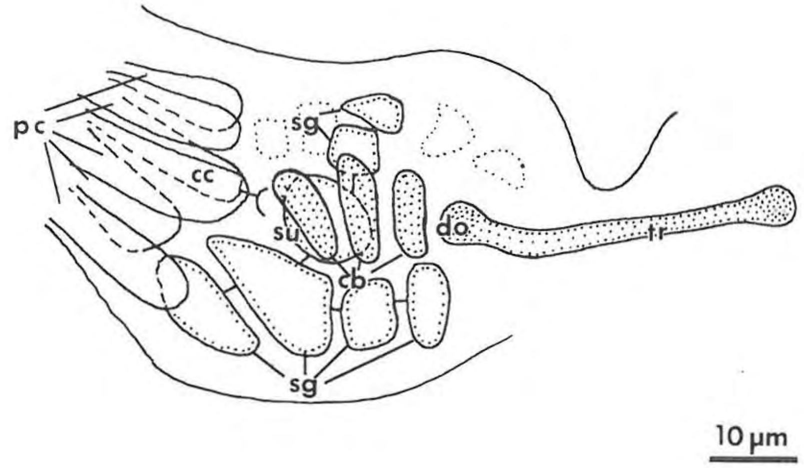
Fig. 13a: Drawing built up from photographs.

Fig.13b and c: Optical sections (a series of photographs focusing from the top down to show various layers of cells).

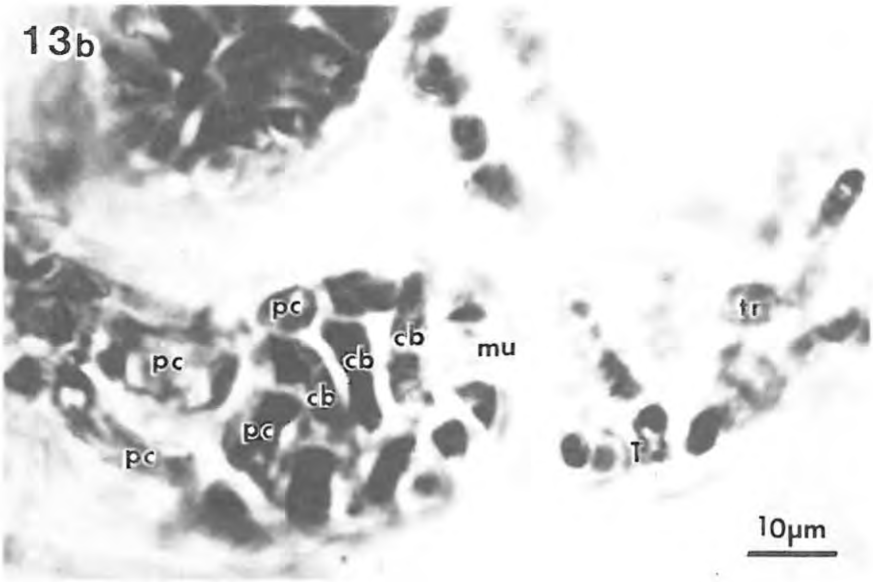
LEGEND:

cb- carpogonial branch cell
cc- central cell
mu- mucilage
p- pit connection
pc- pericentral cell
sg- sterile cell
su- supporting cell
T- trichoblast
tr- trichogyne

13a


pc
sg
cc
su
cb
do
tr
sg
10 µm

13b


pc
cb
mu
tr
pc
cb
cb
pc
pc
T
10µm

13c

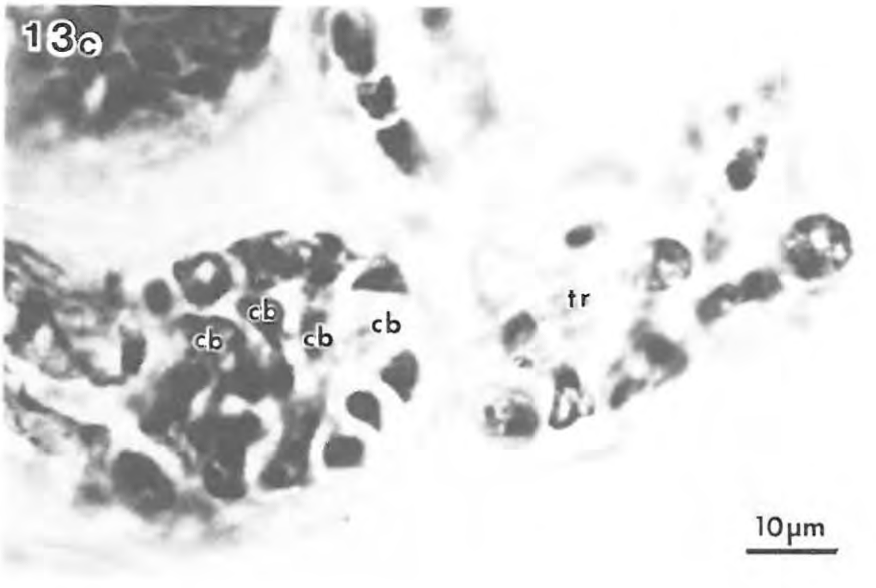

cb
tr
cb
cb
cb
10µm

Fig. 13d-f: Young post-fertilisation cystocarp. Optical sections continued.

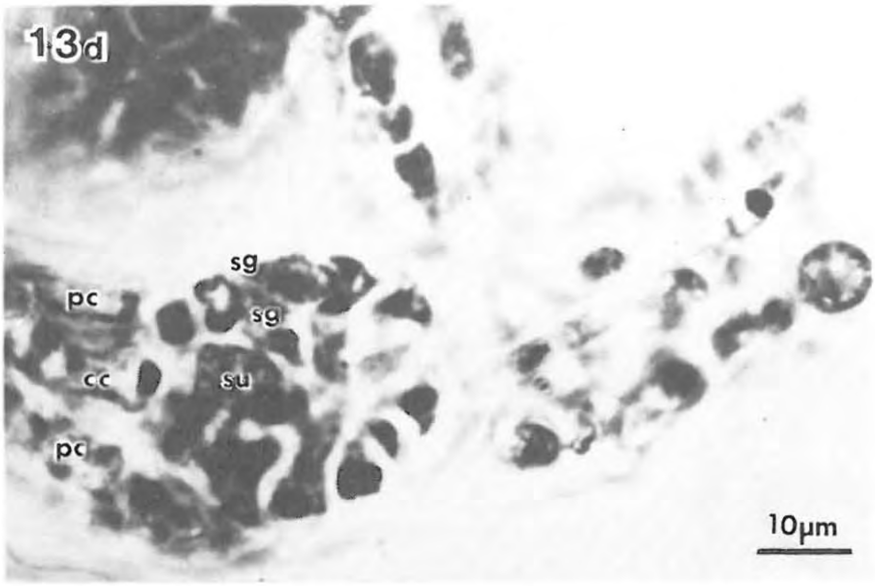
LEGEND:

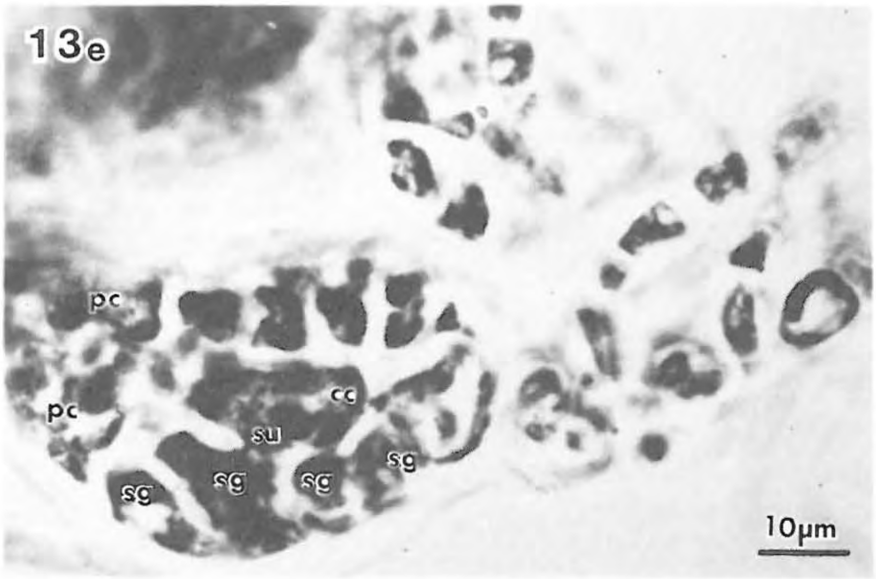
cc- central cell

pc- pericentral cell

sg- sterile cell

su- supporting cell


13d
sg
pc
sg
cc
su
pc
10µm


13e
pc
cc
pc
su
sg
sg
sg
sg
sg
10µm

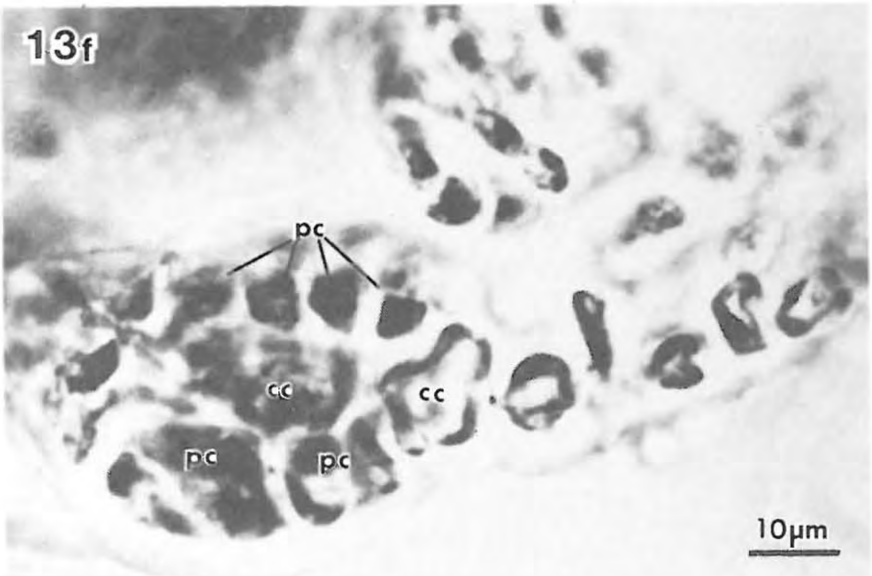

13f
pc
cc
cc
pc
pc
10µm

Fig. 14: Young post-fertilisation stage in cystocarp development.

Fig. 15: Post-fertilisation stage in cystocarp development.

Fig. 15a: Drawing built up from photographs.

Fig. 15b: Optical section.

LEGEND:

au- auxiliary cell

cb- carpogonial branch cell

go- gonimoblast cell

mu- mucilage

p- pit connection

pc- pericentral cell

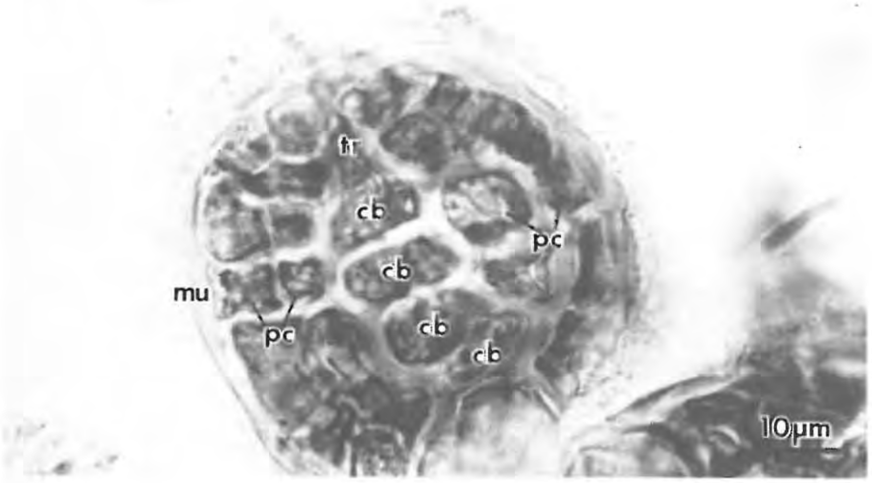
pe- pericarp

sg- sterile cell

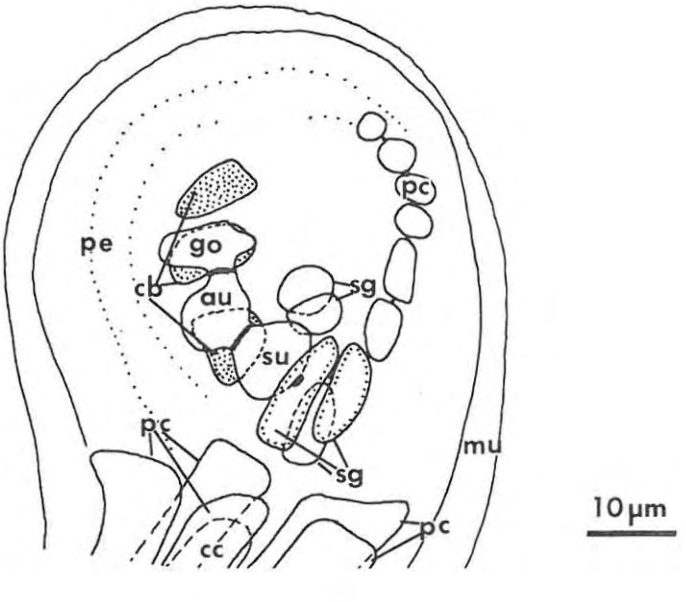
su- supporting cell

tr- trichogyne

14


tr
cb
pc
cb
mu
pc
cb
cb
10µm

15a


pc
go
pe
cb
sg
au
su
pc
sg
mu
10µm
cc
pc

15b

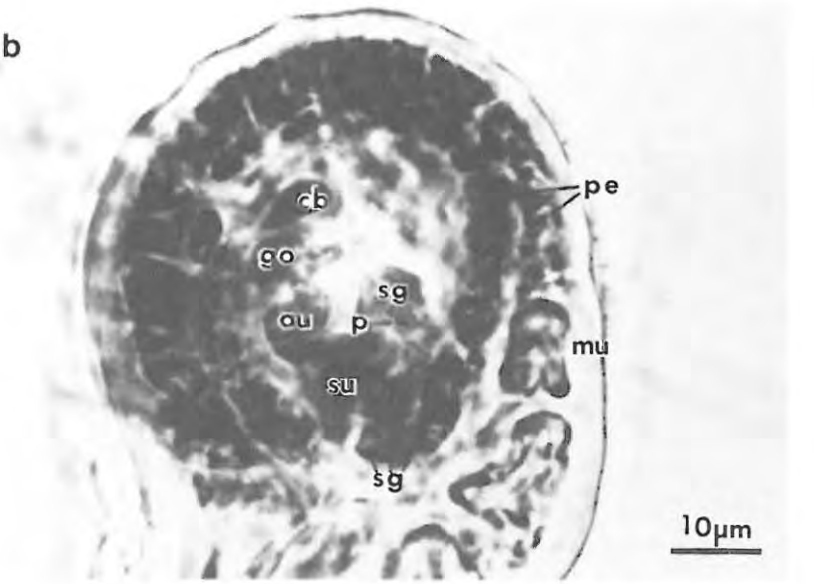

pe
cb
go
sg
au
p
mu
su
sg
10µm

Fig. 15c and d: Post-fertilisation stage in cystocarp development. Optical sections continued.

Fig. 16: Late post-fertilisation stage in cystocarp development.

Fig. 16a: Drawing built up from photographs.

LEGEND:

au- auxiliary cell

cb- carpogonial branch cell

cc- central cell

go- gonimoblast cell

mu- mucilage

os- ostiole

p- pit connection

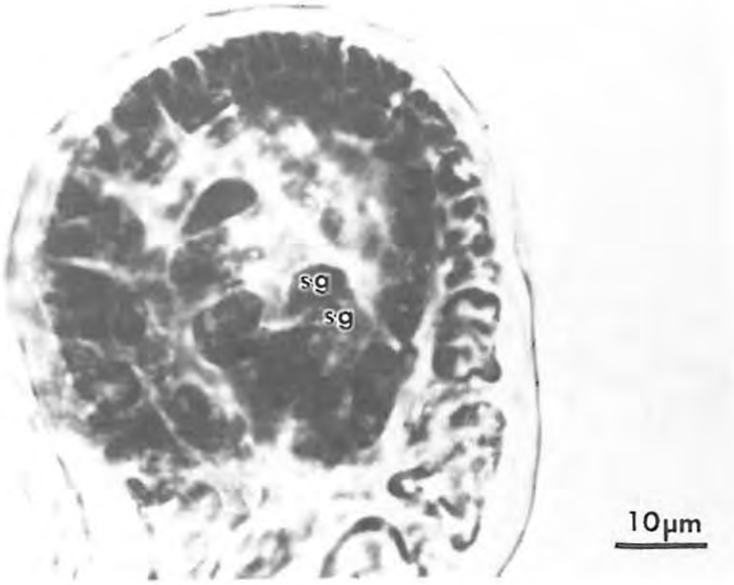
pc- pericentral cell

pe- pericarp

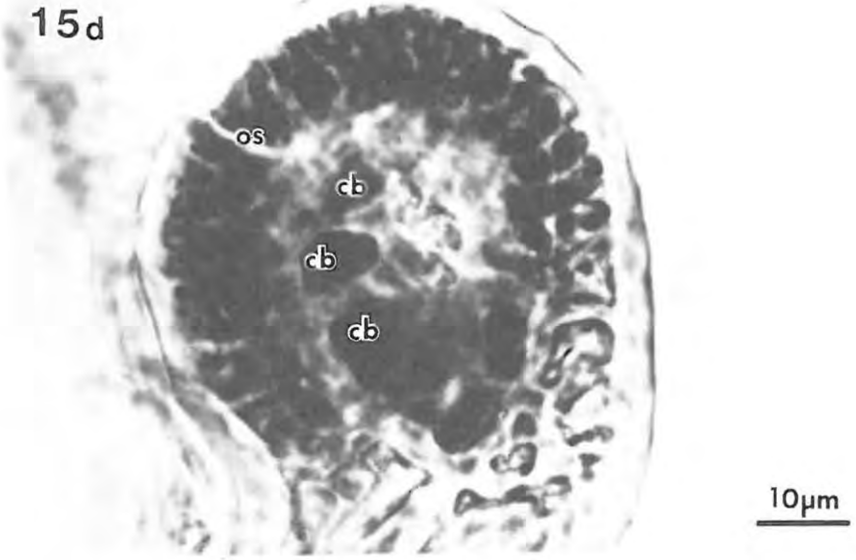
sg- sterile cell

su- supporting cell

15c



15d



16a

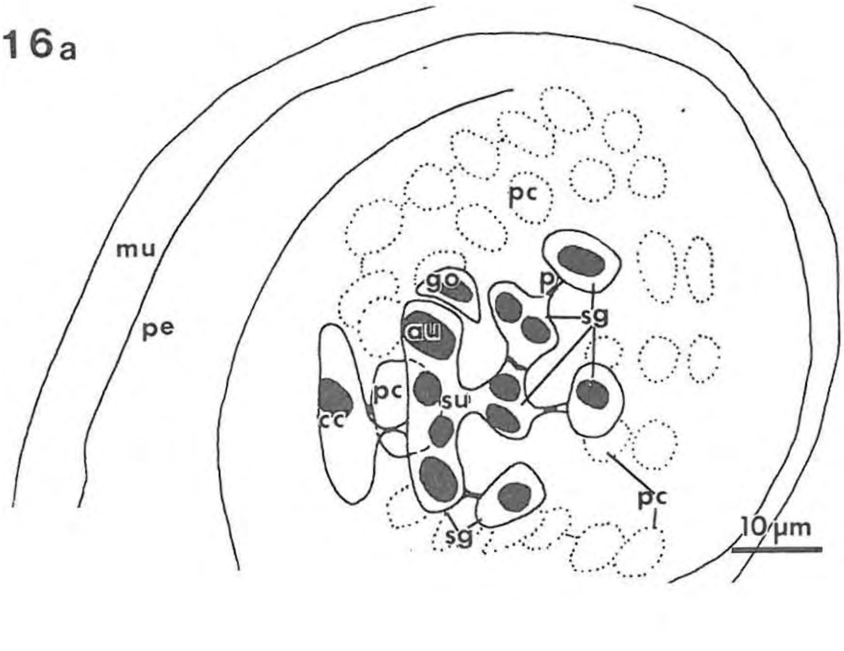
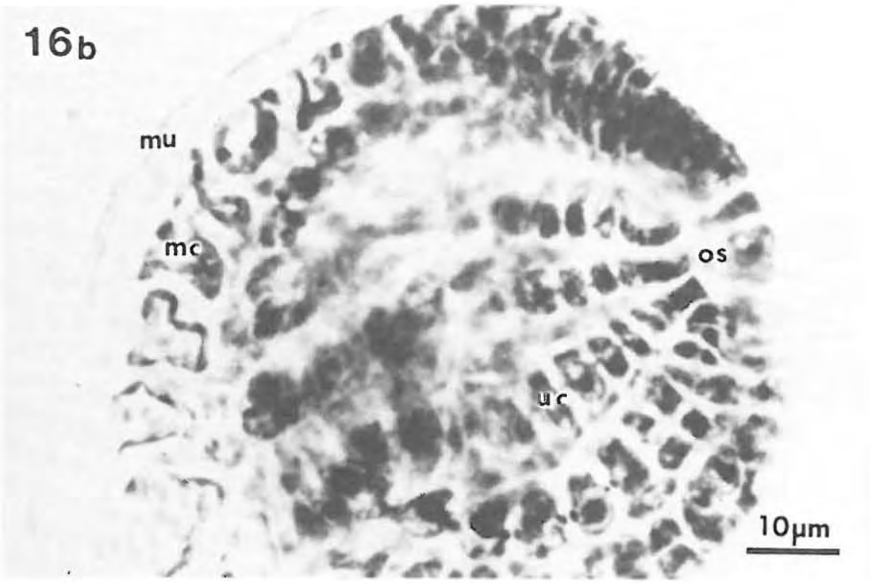


Fig. 16b-d: Late post-fertilisation stage in cystocarp development. Optical sections.

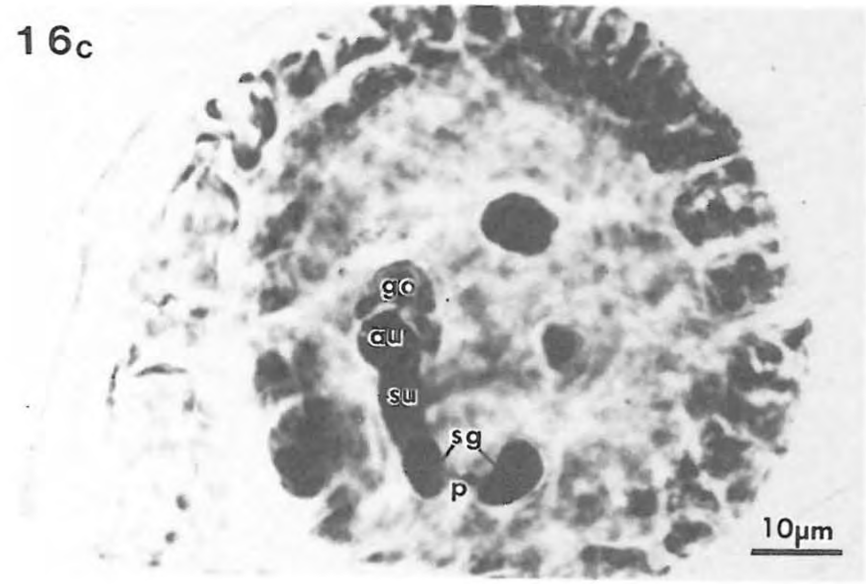
LEGEND:

au- auxiliary cell
cc- central cell
go- gonimoblast cell
mc- multinucleate cell
mu- mucilage
os- ostiole
p- pit connection
sg- sterile cell
su- supporting cell
uc- uninucleate cell

16b



16c



16d

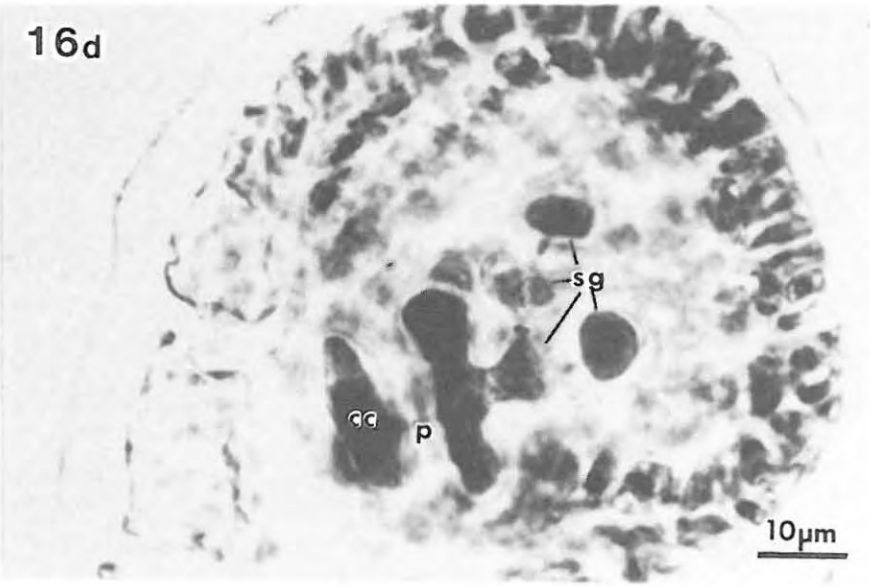


Fig. 16e-g: Late post-fertilisation stage in cystocarp development.
Optical sections continued.

LEGEND:

au- auxiliary cell

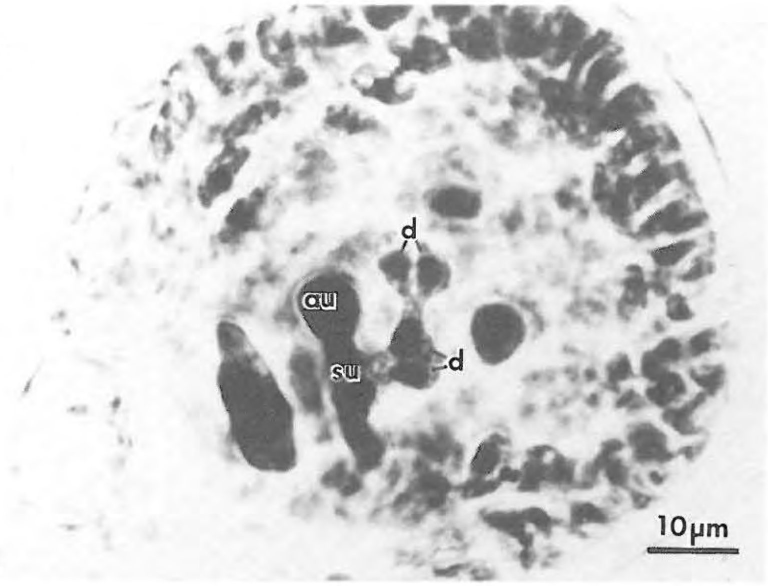
cc- central cell

d- dense spherical regions

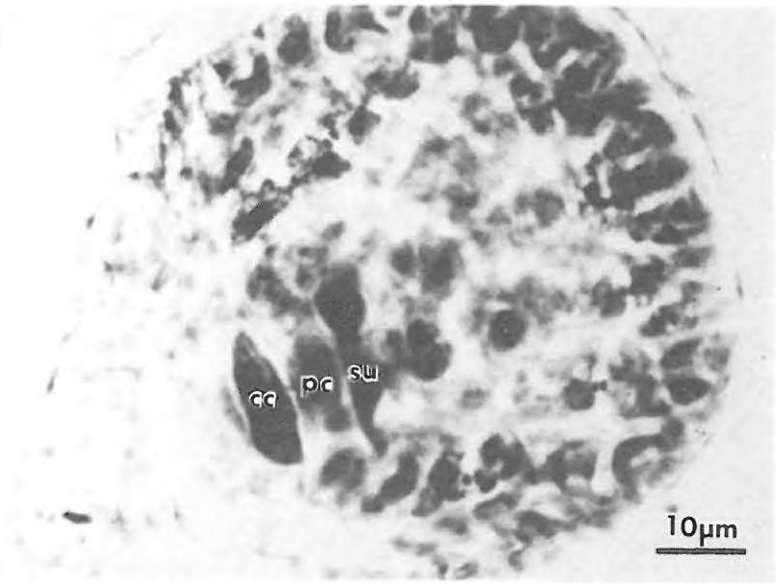
pc- pericentral cell

su- supporting cell

16e



16f



16g

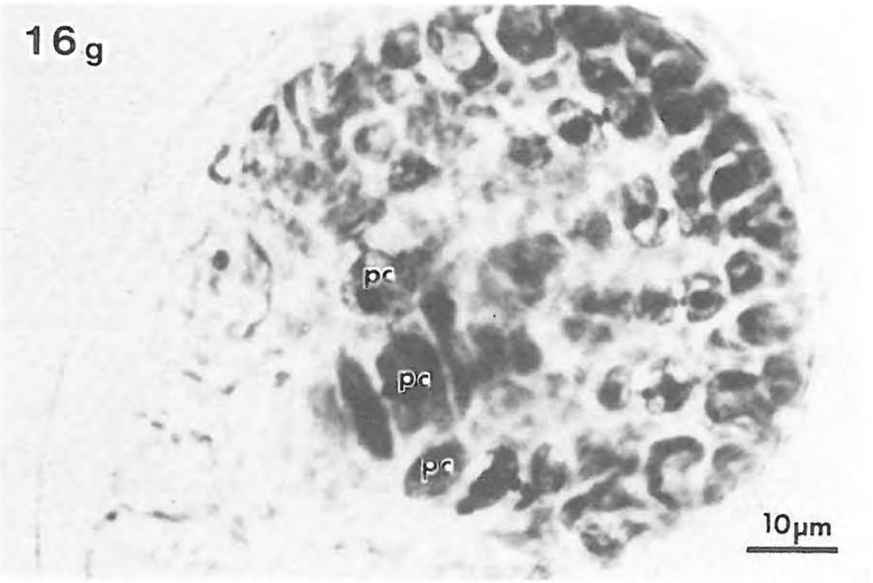


**Fig. 17:** Erect reproductive branch with an advanced stage of cystocarp development where the trichoblasts have disappeared.

**Fig. 18:** Longitudinal section of a mature cystocarp.

**Fig. 19:** Young, erect, male, polysiphonous reproductive branches.

**LEGEND:**

bs- basal segment

cs- carpospore

cy- cystocarp

E- erect reproductive branch

f- fusion cell

go- gonimoblast cell

mu- mucilage

N- nucleus

os- ostiole

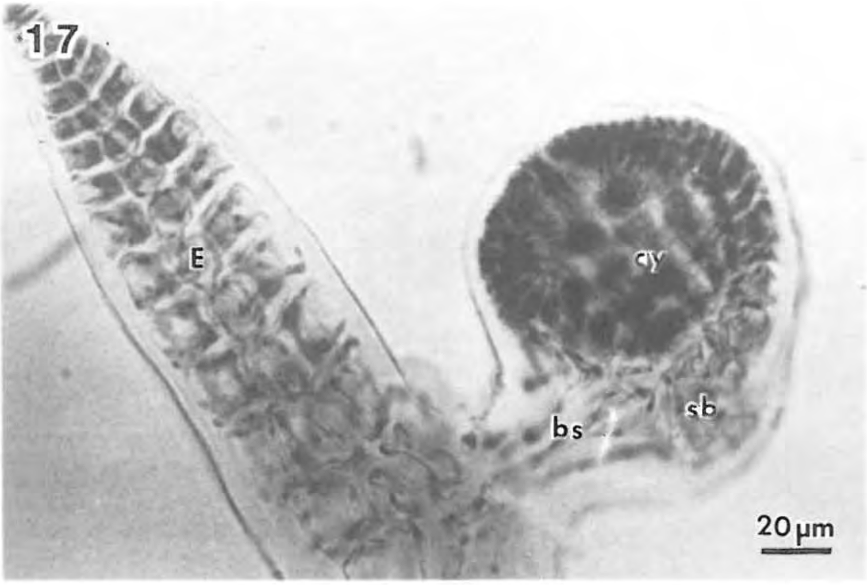
pe- pericarp

sb- suprabasal segment

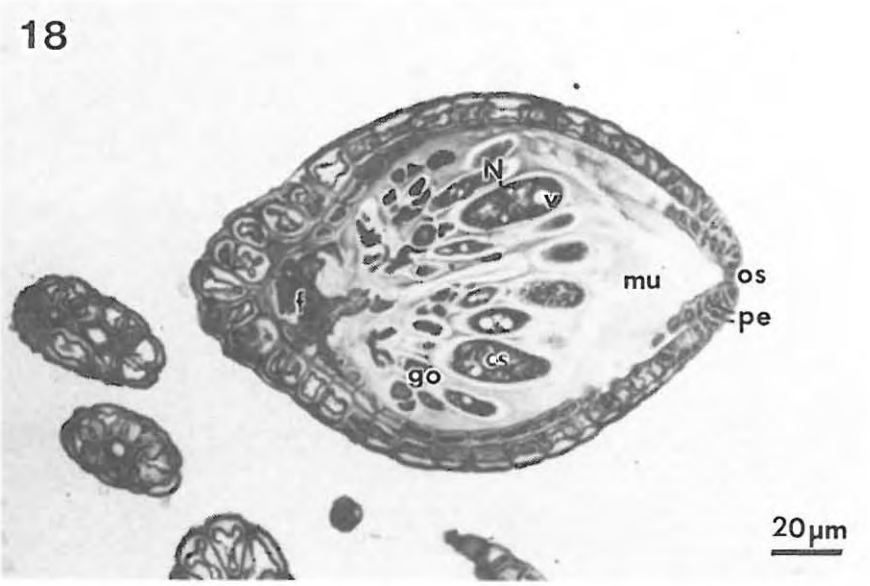
T- trichoblast

v- vacuole

17



18



19

Fig. 20: Sections of mature spermatangial branches.

Fig. 21: Longitudinal section of a mature spermatangial branch.

Fig. 22: Mature tetrasporangial branches.

LEGEND:

a- apical cell

bc- basal cell

cc- central cell

E- erect reproductive branch

p- pit connection

sp- spermatium

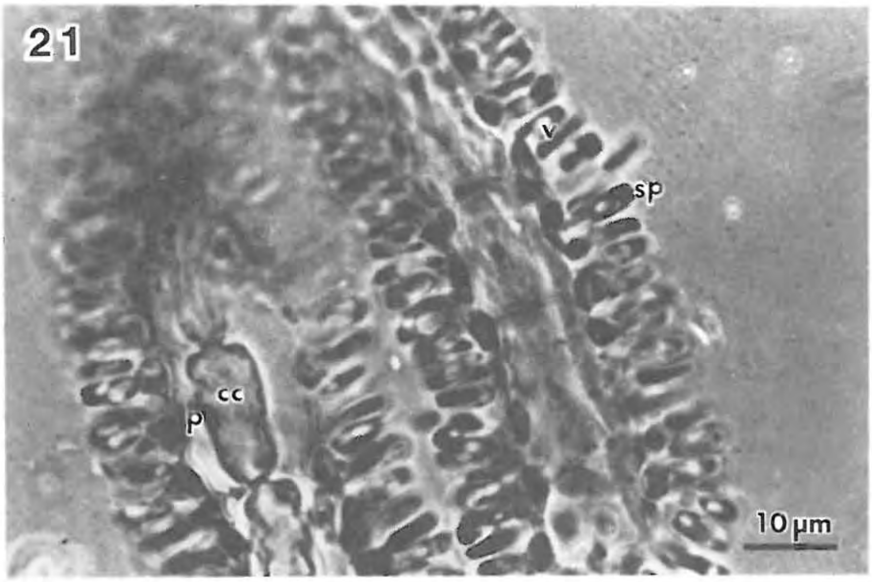
T- trichoblast

tg- tetrasporangium

v- vacuole

20

21



22

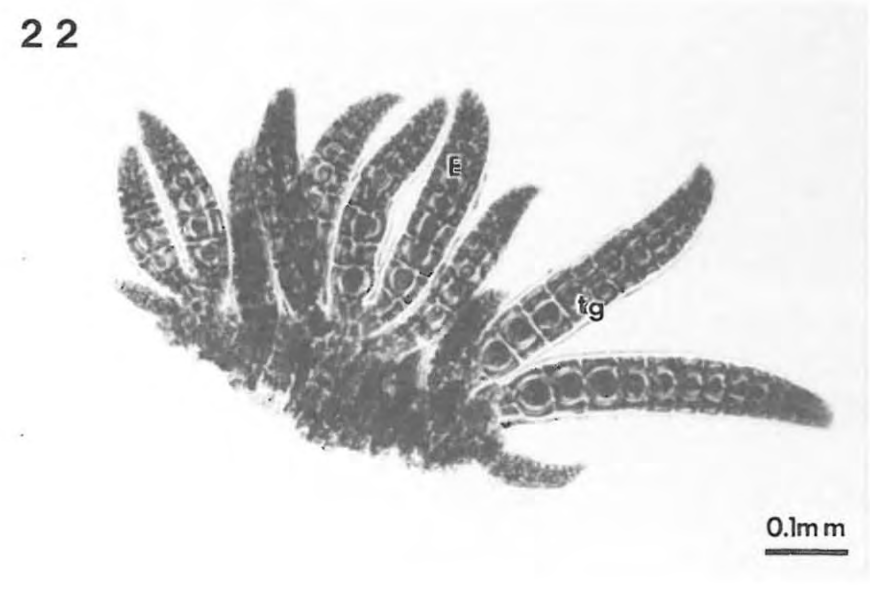


**Fig. 23:** Optical section of a mature tetrasporangial branch.

**Fig. 24:** Cryo-SEM of a cross section through a mature tetrasporangial branch.

**Fig. 25:** Longitudinal section of a mature tetrasporangial branch. Drawn with the aid of a camera lucida.

**LEGEND:**

a- apical cell

cc- central cell

cf- cleavage furrow

co- cover cell

fp- fertile pericentral cell

mu- mucilage

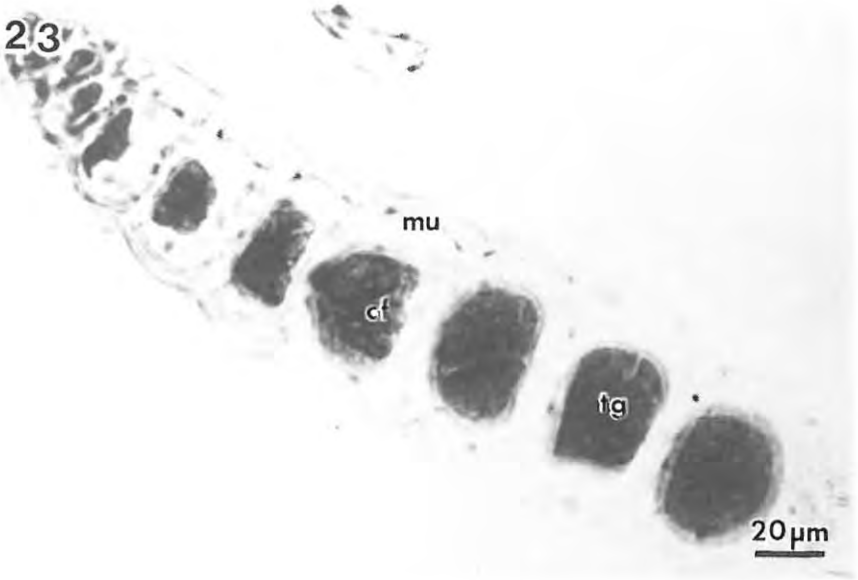
pc- pericentral cell

sc- stalk cell

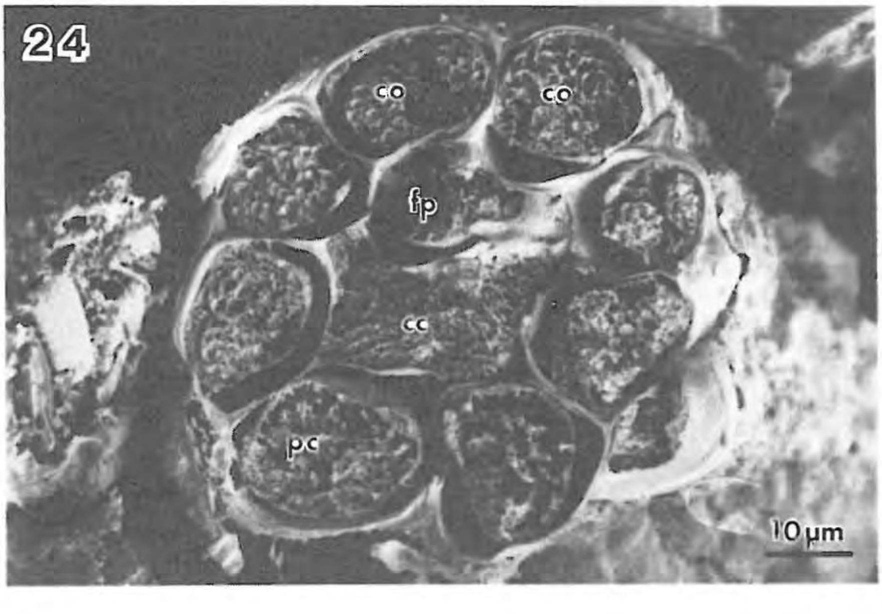
te- tetraspore

tg- tetrasporangium

23



24



25

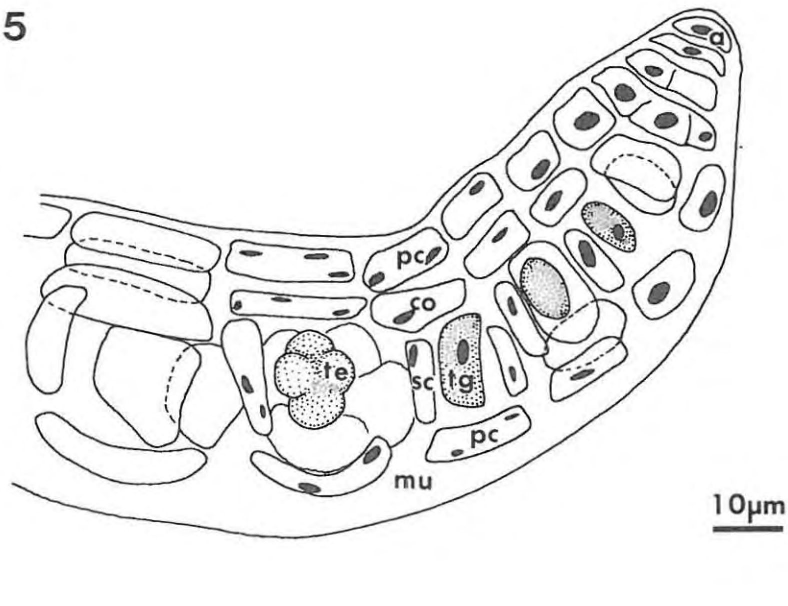


Fig. 26: Cross section of a mature tetrasporangium.

Fig. 27a and b: Carpospores being released from the cystocarp.

LEGEND:

cf- cleavage furrow

cs- carpospore

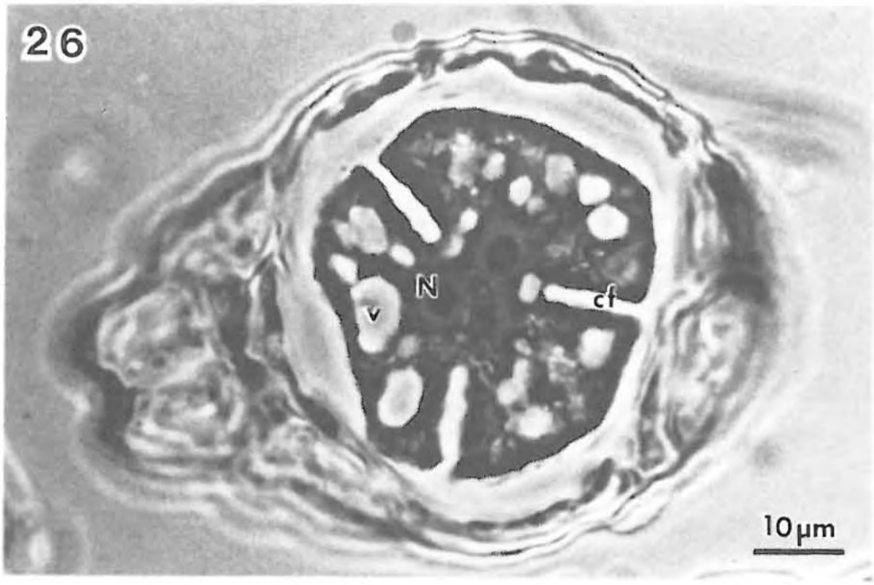
cy- cystocarp

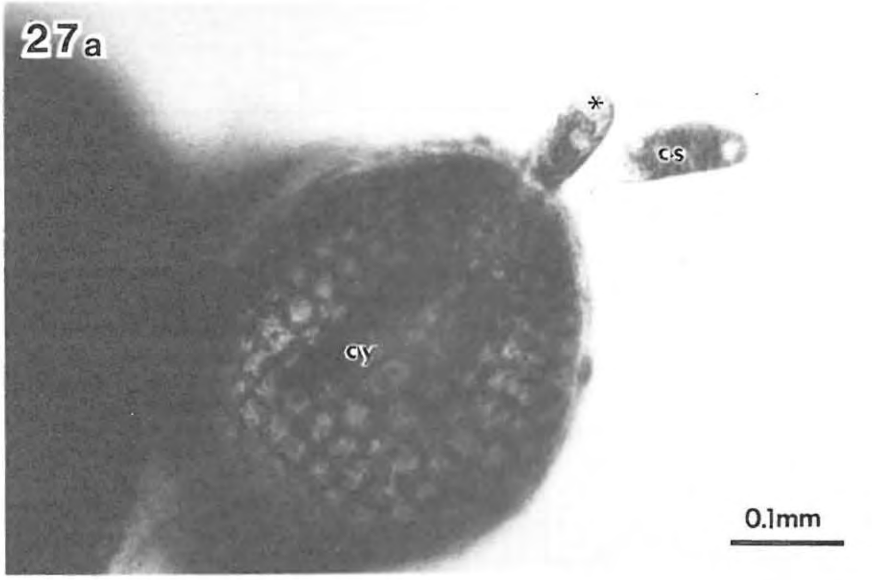
N- nucleus

os- ostiole

v- vacuole

*- clear area


26
N
v
cf
10 µm


27a
*
cs
cy
0.1mm

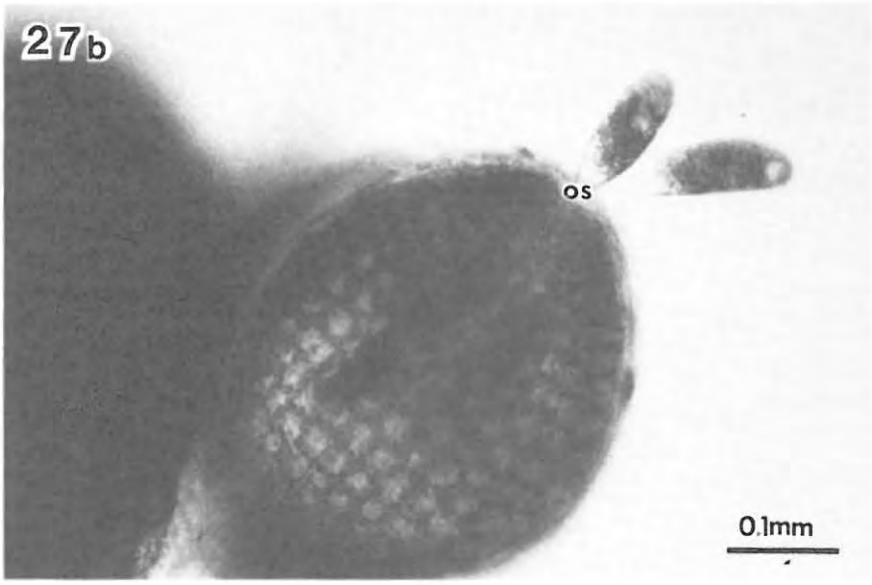

27b
os
0.1mm

Fig. 27c and d: Carpospores being released from the cystocarp.

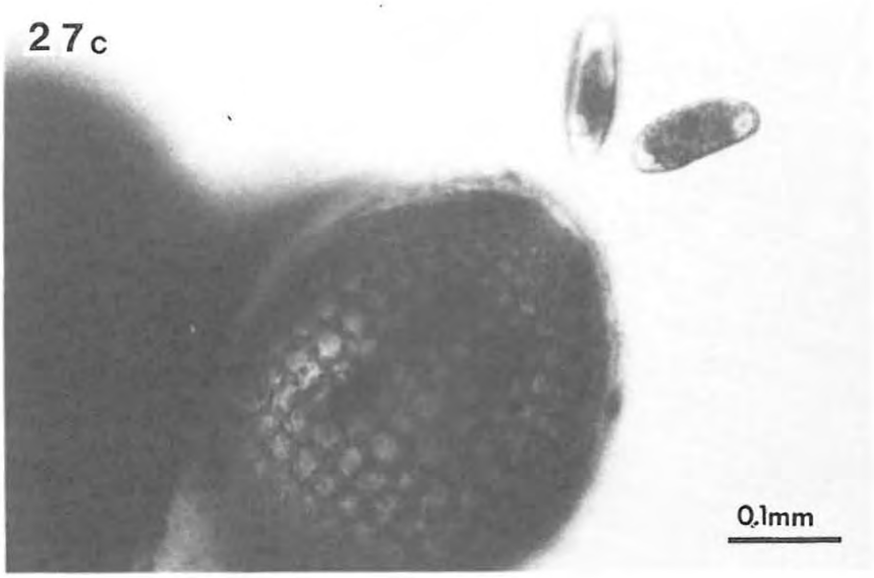
Fig. 28: 24 hour old sporelings and newly released spores.

LEGEND:

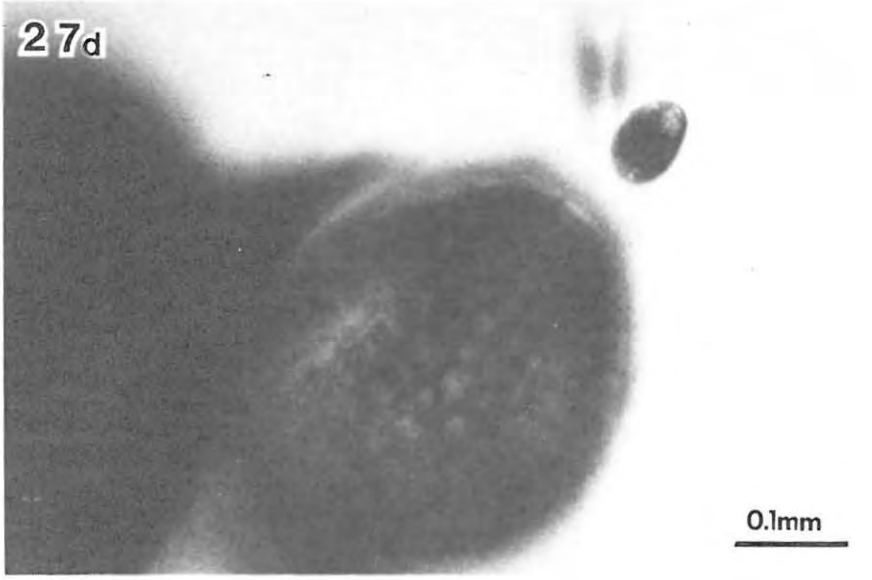
s- newly released spore

*- clear area

27c



27d



28

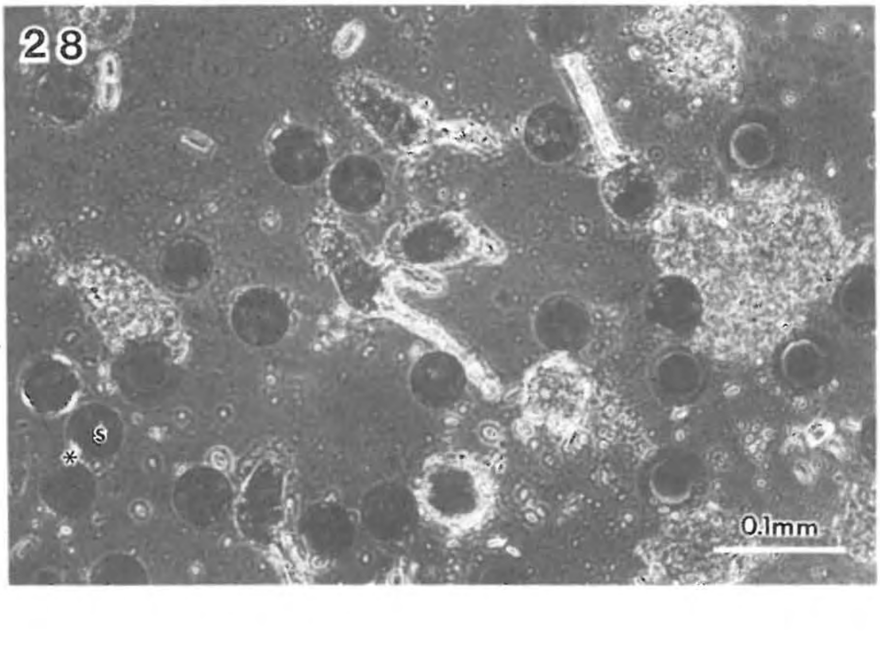


Fig. 29: 3 day old released spores and sporelings.

Fig. 30: 10 day old sporelings.

Fig. 31: Malformed sporeling.

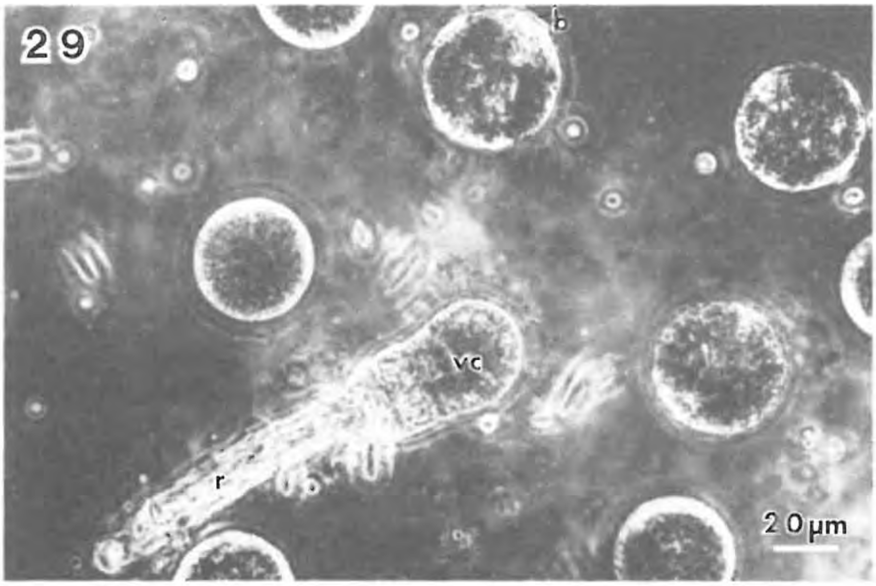
LEGEND:

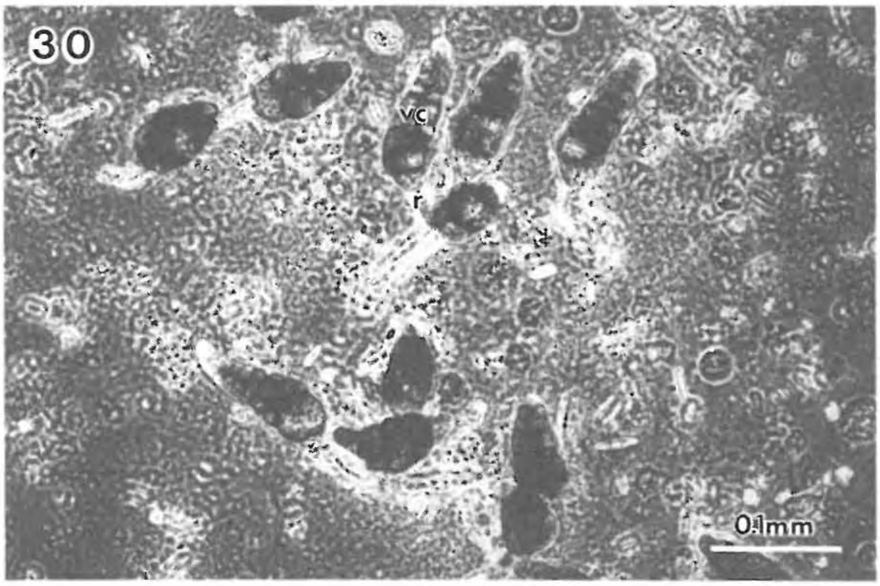
b- colourless bump

lb- lateral bud

r- rhizoid

vc- vegetative shoot


29
b
vc
r
20 µm


30
vc
r
0.1mm

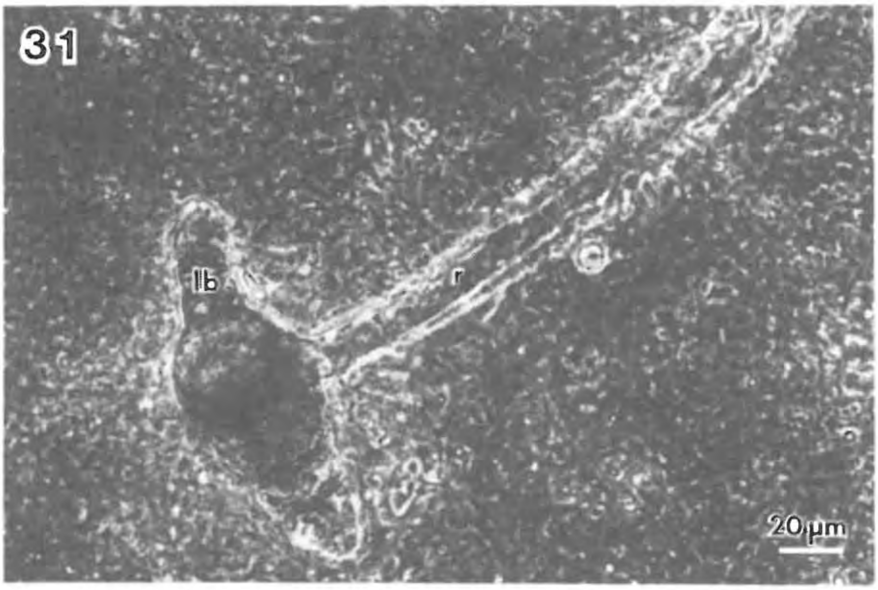

31
lb
r
20µm

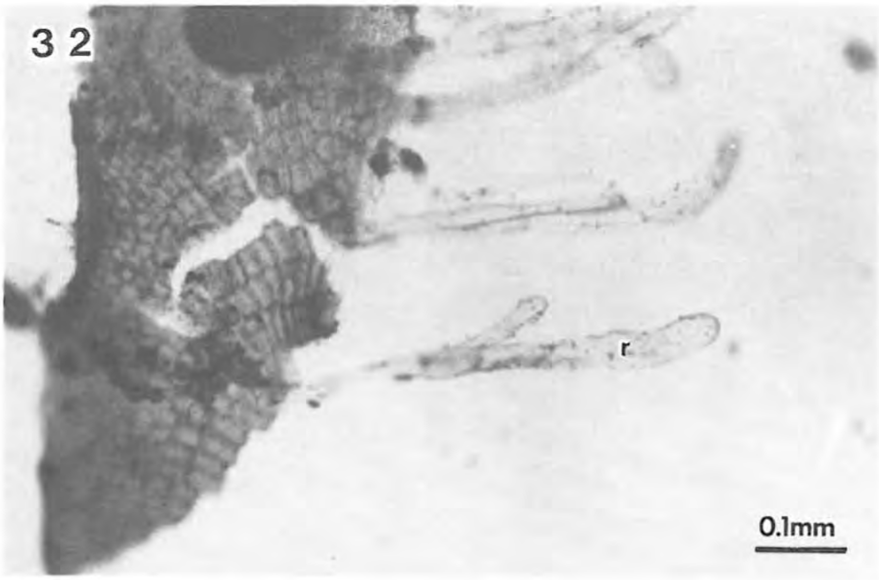
Fig. 32: Rhizoids of a mature thallus which was gently pulled off a *Codium* basiphyte.

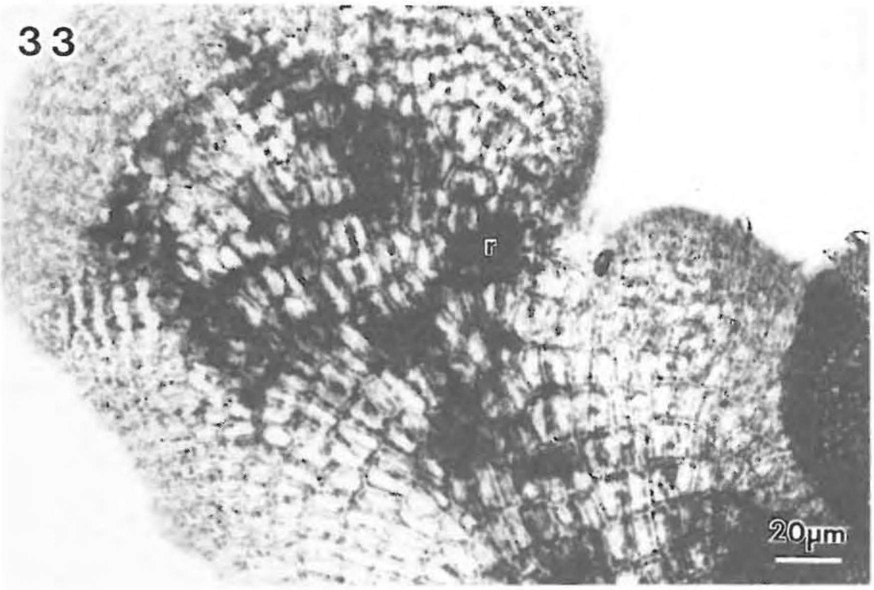
Fig. 33: Rhizoids of a mature thallus showing as dark scattered areas on the ventral surface.

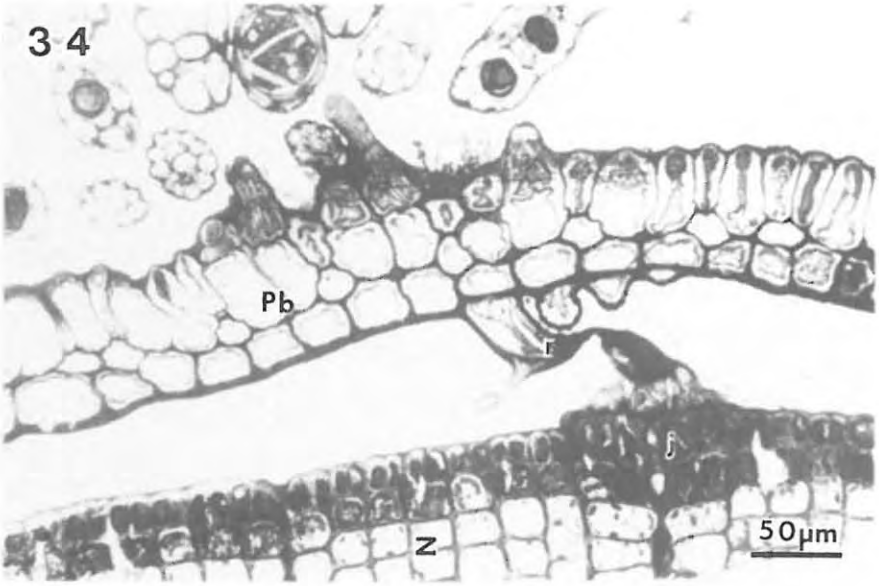
Fig. 34: Cross section of a mature tetrasporophyte. A rhizoid is shown forming a cushion on a *Zonaria* basiphyte.

LEGEND:

Pb- *Placophora binderi*
j- "joint" area on *Zonaria*
r- rhizoid
Z- *Zonaria*


3 2
r
0.1mm


3 3
r
20μm


3 4
Pb
r
j
Z
50μm

# CHAPTER 4

# ULTRASTRUCTURE

## 4.1 INTRODUCTION

Ultrastructural studies within the Florideophycideae indicate that most species conform to a basic pattern both in the vegetative and reproductive thalli.

### The Vegetative Thallus

#### a) The Cell Covering

A plasmalemma surrounds the cell contents. This is often invaginated and may act in nutrient uptake or indicate an active cell wall synthesis by the fusion of vesicles with the plasmalemma and release of their contents to the cell wall (Dodge 1973). External to the cell wall is a thick mucilage layer containing cellulose microfibrils in an amorphous muco-polysaccharide matrix (Bouck 1962; Dodge 1973; Duckett and Peel 1978; Kugrens and West 1973a,b). The exact appearance of the cell wall depends on cell type and thallus area (Duckett and Peel 1978). The extreme outer layer of the mucilage investment is often seen as an electron dense "cuticle" which has a strong affinity for protein and pectic stains (Bisalputra et al 1967; Bold and Wynne 1978; Dixon 1973; Dodge 1973).

b) Photosynthetic Apparatus

Chloroplasts, which differ from those of higher plants and other algal groups, are a distinctive feature of the "red algae" (Fig.35).

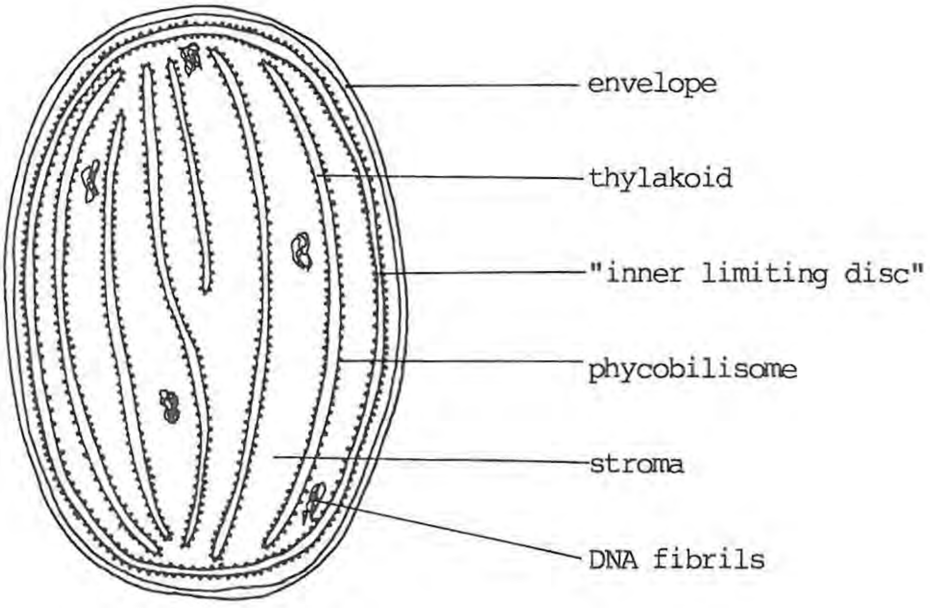


Fig.35: Diagrammatic representation of a "red algal" chloroplast.

A typical double membrane chloroplast envelope occurs. Single, unstacked thylakoids usually lie parallel to the long axis of the chloroplast. One thylakoid often runs parallel to the envelope as an "inner limiting disc" from which other thylakoids may arise (Bouck 1962; Dodge 1973; Duckett and Peel 1978). Small electron dense spheres, the phycobilisomes, occur on the thylakoid surfaces. The stroma contains ribosomes and, in most species, electron transparent areas with irregularly arranged DNA microfibrils (Bisalputra and Bisalputra 1967; Dodge 1973; Tripodi 1971). The size, shape and number of the chloroplasts per cell and thylakoid distribution vary according to species and cell type. In young cells chloroplasts often contain only the stroma and an "inner limiting disc" (Bouck 1962; Duckett and Peel 1978).

Pyrenoids are not usually found in members of the Florideophycideae (Bold amd Wynne 1978).

### c) The Nucleus

Typical eukaryotic nuclei are present with double membranes, nuclear pores and one to several nucleoli (Bouck 1962; Dodge 1973). The chromatin appears as finely filamentous or granular euchromatin and a more densely granular heterochromatin. Mitosis and meiosis are thought to occur in a similar fashion to that in higher plants and animals. Direct evidence for this is lacking in many species as chromosomes are usually very small, making accurate observations difficult (Brown and Weier 1970; Dixon 1966; Dodge 1973; Duckett and Peel 1978; Leedale 1970; McDonald 1972; Scott et al 1980). Synaptonemal complexes, such as those associated with mitosis and meiosis in higher organisms, have been observed in the reproductive cells of some species indicating normal division processes for these algae (Kugrens and West 1972a; Peyriere 1974).

### d) Storage Products

The major storage product is a polysaccharide reserve, similar to the starch of higher plants, called floridean starch. This occurs as variously shaped granules scattered in the cytoplasm (Bold and Wynne 1978; Sheath et al 1981). Unlike higher plants the chloroplasts are not responsible for starch synthesis. Borowitzka (1978) claims that the endoplasmic reticulum is responsible as it provides a site and source for the enzymes which are necessary to polymerize hexose to starch. Tripodi (1971) and Kugrens and West (1973a) propose a dictyosome formation, although no real evidence exists to support this theory.

### e) Mitochondria, Endoplasmic Reticulum and Vacuoles

Typical mitochondria with double membranes, cristae, grana and DNA fibrils occur. Both rough and smooth endoplasmic reticulum occurs in most cells (Bouck 1962; Brown and Weier 1970; Dodge 1973).

Vacuoles are limited by a tonoplast and contain either clear or fibrillar contents. They tend to increase in size with age and their shape varies according to organelle density (Bouck 1962).

Vacuoles appear first as expansions of endoplasmic reticulum and are later enlarged by the addition of Golgi derived vesicles (Brown and Weier 1970).

### f) Golgi Apparatus

Golgi apparatus is a collective term for all the dictyosomes which may occur within a cell (Mollenhauer and Morré 1966). In "red algae" dictyosomes commonly occur as polar bodies with a forming face associated with mitochondria and a mature face associated with vesicles (Brown and Weier 1970; Kugrens and West 1973a; Ramus and Robins 1975). The function of the Golgi apparatus is generally said to be cell wall synthesis and vacuole manufacture. Brown and Weier (1970) recognised two dictyosome types, one where the mature face is directed towards the cell wall, producing vesicles which add to the wall material, and another type where the mature face is directed inwards with vesicles forming vacuoles.

Another proposed function of dictyosomes is that of storage product synthesis in vegetative cells (Tripodi 1971) and in reproductive cells (Chamberlain and Evans 1973; Dodge 1973; Kugrens and West 1973a,1974).

### g) Pit Connections

These structures are unique to the Florideophycideae. Primary pit connections are found between cells of the same chain which have divided from the apical cell (Fritsch 1935) and in older thallus regions; secondary pit connections may form between cells of adjacent chains (Bold and Wynne 1978). Under the light microscope they are seen as small dense structures between cells (Fig.36).

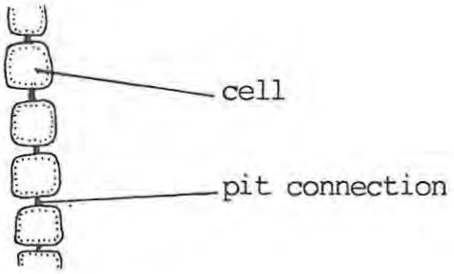


**Fig.36:** Pit connection as seen with a light microscope.

At the ultrastructural level pit connections generally consist of a plug core, a plug cap and a plug membrane (Fig.37).

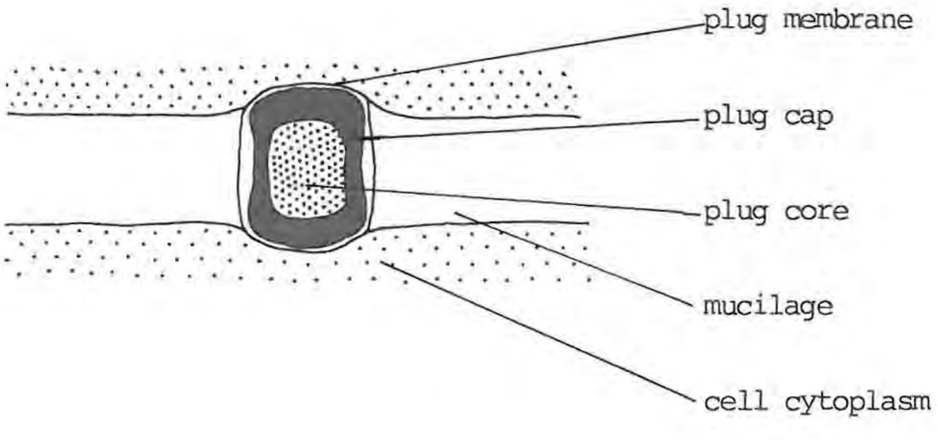


**Fig.37:** Pit connection as seen by electron microscopy.

The plug core forms the pit itself and consists of a homogeneous electron dense granular matrix traversing the cell wall and usually protruding as convex domes into the cell cytoplasm (Pueschel 1977). It may be proteinaceous in structure (Bisalputra et al 1967; Pueschel 1980a; Ramus 1971; Tripodi 1971).

The plug cap is a thin electron dense layer covering the surface of the plug core (Lee 1971; Pueschel 1977). Pueschel and Cole (1982) have classified the pit connections into three groups based on the number of plug cap layers which may be present.

The plug membrane, also referred to as the cap membrane (Quirk and Wetherbee 1980; Wetherbee and Scott 1980), is a thin membrane external to the plug cap and is usually formed by the plasmalemma. Its permanency is questionable as it is not always present and in some instances appears to be in the process of being either formed or broken down (Wetherbee and Scott 1980).

Pit connections may form as a result of incomplete furrowing of the plasmalemma during the final stage of cytokinesis (Aghajanian and Hommersand 1978; Pueschel 1977). In Ceramium diaphanum (Hawkins 1972) and Pseudogloiophloea (Ramus 1969b) the pit connections were formed by an ingrowth of the cell walls where vesicles of either dictyosome or endoplasmic reticulum origin accumulated and condensed to form the plug core. In Batrachospermum sirodotii (Aghajanian and Hommersand 1978) the plasmalemma between two newly divided cells remained incompletely divided and endoplasmic reticulum derived vesicles accumulated and polymerized to form the plug core in this area.

The function of the pit connection is a strongly debated issue. The most popular proposed function is that of nutrient transport. Bisalputra et al (1967) claim that continuity across the pit connection is difficult to envisage and Ramus (1969a; 1971) reinforces this by saying that the presence of the plug membrane would further disrupt this continuity. Most authors feel that although the pit connection looks like a plug and is homogeneously proteinaceous, which would exclude a transport function, it is the only means of transport where plasmodesmata do not occur and where the cell wall itself is extremely thick and homogeneous (Evans et al 1973; Hawkins 1972; Kugrens and West 1973b; Pueschel 1977; Ramus 1971; Turner and Evans 1978; Wetherbee and Scott 1980). Some proof of transport via the pit connections is seen in Ceramium diaphanum where the plug core enlarges after its formation indicating transport of material across the plug cap (Hawkins 1972). Some authors see the pit connection as an area of preformed weakness which may disintegrate at times when intercommunication is required (Aghajanian and Hommersand 1978; Ramus 1971).

The name "pit connection" is said to be misleading by some authors as it is neither a "pit" nor a "connection" (Ramus 1971) although, when apparent cellular continuity occurs across the structure, the name would be appropriate (Pueschel and Cole 1982). Wetherbee (1979) refers to the structure as a "transfer connection" but only when it is morphologically distinct and where transport or communication appears necessary or where outside nutrition is relied on (Quirk and Wetherbee 1980). In either case a function is still presumed. Bisalputra _et_ _al_ (1967) refers to the opening in the wall as the "aperture" and the plug-like structure as the "plug" which precludes a function.

## The Reproductive Thallus

### a) The Male Structures

According to classical terminology, the pericentral cells divide to produce spermatangial mother cells which divide to form spermatangia in which spermatia are produced (chapter 1). Kugrens (1980) refers to these mother cells as spermatial mother cells rather than spermatangial mother cells because the product of mother cell cleavage is a spermatium, not a spermatangium. In addition, the wall layers which occur around each male gamete are present at the time of release and are not left behind in _Levringiella gardneri_ and _Erythrocystis saccata_ (Kugrens and West 1972b) nor in _Polysiphonia hendryi_ (Kugrens 1980). In these two studies the product of cleavage from the mother cell is released as the male gamete and is therefore the spermatium. Kugrens (1980) and Kugrens and West (1972b) believe that the classical terminology holds only in cases where a single spermatium with its own wall is enclosed within another wall (the spermatangial wall) which is left behind at spermatial release. In the following discussion the classical terminology is adhered to.

Spermatangia, newly cleaved from the spermatangial mother cells, are avacuolate and non polar. They possess few mitochondria, some plastids, a distal nucleus with respect to the spermatial mother cell, many ribosomes, a few dictyosomes and an extensive endoplasmic reticulum with a few inflated cisternae filled with a granular matrix. The latter appear to coalesce into small semi-electron dense granular structures near the spermatangial mother cell (Fetter and Neushul 1981; Kugrens 1974,1980; Kugrens and West 1972b; Peyrière 1971,1974; Scott and Dixon 1973b). A short while later, dictyosome activity increases and may initiate wall deposition by vesicles releasing material to the wall (Kugrens and West 1972b; Scott and Dixon 1973b). As spermatia mature, wall deposition continues and pit connections are either severed or remain intact (Kugrens 1980). Simultaneously with wall deposition one or two large vesicles with fibrillar contents form in the proximal half of the spermatium. These are the spermatial vesicles and are a distinctive feature of all spermatia, differing only in size and appearance. The initial formation of the spermatial vesicles may be by the coalescence of:

a) endoplasmic reticulum vesicles (described above) with dictyosome vesicles added later (Fetter and Neushul 1981; Kugrens 1974). This is substantiated by the early inactivity of the dictyosomes and the production of two vesicle types by the dictysomes, one producing wall material and the other spermatial vesicle material (Fetter and Neushul 1981).

b) dictyosome derived vesicles (Peyrière 1971,1974) perhaps with some endoplasmic reticulum involvement (Scott and Dixon 1973b).

c) endoplasmic reticulum derived vesicles alone as dictyosomes form after spermatial vesicles have developed (Kugrens 1980; Kugrens and West 1972b).

The differences observed above are probably explained by the number of species used in those studies. At maturity the spermatial vesicles may fuse if more than one is present, usually becoming extracytoplasmic (Fetter and Neushul 1981; Kugrens 1974; Kugrens and West 1972b; Scott and Dixon 1973b).

Released spermatia usually contain a single nucleus with condensed or dispersed chromatin and no nucleoli, small starch grains, a few mitochondria, proplastids, dictyosomes, endoplasmic reticulum and a mucilage wall of varying thickness (Kugrens 1974; Kugrens and West 1972b,1974; Scott and Dixon 1973b).

## b) The Female and Tetrasporic Structures

Reproduction in female and tetrasporic thalli results in the production of spores which follow the same developmental pattern with similar ultrastructural features.

The early developmental stages differ in that the female undergoes a complex postfertilisation development (chapter 1) before spore production, while in the tetrasporic thalli the spores are cut off from the tetrasporangial mother cells early in the development and resemble young vegetative cells at the ultrastructural level (Chamberlain and Evans 1973; Hawkins 1974a,b; Kugrens and West 1972c; Peyrière 1974; Pueschel 1979,1982; Scott and Dixon 1973a).

In the female the trichogyne possesses all the usual organelles. Pit connections occur between carpogonial branch cells before the fusion cell forms. Gonimoblast filaments are usually lightly pigmented, lack starch grains and possess nuclei (Wetherbee 1980). The fusion cell is irregularly shaped and enlarges as the carposporophyte matures by the fusion of other cells with it (chapter 1). No metabolic functions are associated with the fusion cell as few significant organelles occur within it. Kugrens and Arif (1981) postulate that it may act as:

a) an early barrier against haploid interference from other cells,

b) a large nutrient store for the developing gonimoblast and spores,

c) a source of mucilage which forms a large part of the fusion cell and, later on, the cystocarp cavity.

It is generally agreed that mitosis occurs to produce carpospores from the gonimoblast filaments and that meiosis occurs within newly formed tetraspores to produce four haploid spores. In tetraspore development, cleavage has to occur to separate the four spores. This process starts soon after meiosis and continues simultaneously with tetraspore development. Complete cleavage occurs only at maturity, which may reflect a need for cytoplasmic continuity to synchronize the development of the four spores (Pueschel 1979,1982).

Newly formed carpospores and tetraspores contain a few chloroplasts usually only with the "inner limiting disc", endoplasmic reticulum, mitochondria usually associated with the nucleus, few starch grains if any, dictyosomes with straight or slightly curved profiles and mitochondria at their forming faces, and a large central nucleus with a nucleolus, condensed chromatin and an irregular wall (Alley and Scott 1977; Chamberlain and Evans 1973; Hawkins 1974a,b; Kugrens and West 1972c,1973a,1974; Pueschel 1979,1982; Scott and Dixon 1973a; Triemer and Vasconcelos 1977; Tripodi 1971; Wetherbee 1978; Wetherbee and West 1976,1977; Wetherbee and Wynne 1973). The irregular wall may be due to pressure of adjacent organelles

(Kugrens and West 1973a; Wetherbee and Wynne 1973), or it may reflect an active participation by the nucleus in spore development (Pueschel 1979).

Later stages show additional structures, known as fibrillar vesicles. These form either by dictyosome derived vesicles fusing together (Alley and Scott 1977; Triemer and Vasconcelos 1977; Tripodi 1971; Wetherbee and West 1977) or by swollen endoplasmic reticulum cisternae coalescing and then later being added to by dictyosome derived vesicles (Kugrens and West 1973a,1974). The function of fibrillar vesicles may be storage for later spore wall development (Triemer and Vasconcelos 1979; Kugrens and West 1973a) or simply as a mucopolysaccharide storage body (Tripodi 1971). Spore walls are deposited in early stages by small dictyosome derived vesicles ( Kugrens and West 1972c; Pueschel 1979; Triemer and Vasconcelos 1979).

In some species, later stage spores develop another vesicle type variously called striated, striped or crystalline vesicles (Chamberlain and Evans 1973; Kugrens and West 1972c,1973a,1974; Triemer and Vasconcelos 1977; Tripodi 1977; Wetherbee and West 1977). These have various shapes and sizes and are membrane bound. Contents are organised into alternating lines of electron dense and electron transparent areas giving the striated appearance. An endoplasmic reticulum origin and function as a protein storage body for later stages is generally proposed for these vesicles. Together with the appearance of striated vesicles, dictyosomes become semi-circular in profile and may be contributing to further fibrillar vesicle formation (Kugrens and West 1972c,1973a). The dictyosomes may also produce vesicles which either fuse with the plasmalemma, contributing to the deposition of the spore wall, or they may first fuse with the fibrillar vesicles which themselves fuse with the plasmalemma (Scott and Dixon 1973a; Kugrens and West 1972c). Organelles are all well developed in these intermediary stages including chloroplasts and starch grains.

The final stage of spore development is usually represented by the disappearance of striated vesicles and the appearance of small vesicles with electron dense cores termed cored vesicles. Dictyosomes have straight profiles. The origin of the cored vesicles is unknown and various theories have been proposed. The most popular theory is a dictyosome origin (Alley and Scott 1977; Kugrens and West 1972c,1974; Chamberlain and Evans 1973; Scott and Dixon 1973a; Peyrière 1970; Ramm-Anderson and Wetherbee 1982; Tripodi 1971; Hawkins 1974a,b). Alley and Scott (1977) propose that this occurs by a centripetal accumulation of fibrous material as cisternae mature from the forming face of the dictyosome to produce the core. Hawkins (1974a,b), Ramm-Anderson and Wetherbee (1982) and Scott and Dixon (1973a) propose that the entire cisterna may slough off to produce the vesicle with the central uninflated portion of the cisterna forming the core. Hawkins (1974b) proposes that the cisterna may also envelop a portion of cytoplasm to produce the core. Wetherbee and West (1976,1977) claim that a gradual transition and maturation of the striated vesicles to cored vesicles occurs. Most authors agree that the core is probably proteinaceous while the surrounding area is carbohydrate. The function of these vesicles is also unclear and is variously stated that they:

a) are responsible for overall increase in spore size and wall deposition, thereby facilitating spore release by increasing pressure (Hawkins 1974a,b; Scott and Dixon 1973a).

b) form the final process of cleavage in tetraspores (Scott and Dixon 1973a).

c) produce the adhesive material during spore settlement and germination (Chamberlain and Evans 1973; Wetherbee 1978).

Mature spores commonly contain single central nuclei, floridean starch grains, a few fibrillar vesicles, many cored vesicles, chloroplasts, mitochondria, endoplasmic reticulum and dictyosomes with straight profiles.

Released spores generally show a further reduction of fibrillar vesicles and the gradual disappearance of cored vesicles with time. Cored vesicles have also been seen to be fusing with the plasmalemma, the "core" being released first (Wetherbee 1978).

## 4.2 AIMS

Ultrastuctural studies were carried out on vegetative and reproductive thalli of Placophora binderi to establish if it conformed to the basic patterns described in section 4.1.

These studies also aimed to add to the debated areas of "red algal" ultrastructure such as the structure of pit connections, rhizoid function, spermatial vesicle function and formation in the male thallus, the structure, function and origin of the vesicles formed during sporogenesis in the carposporophyte and tetrasporophyte phases of the life history.

## 4.3 RESULTS

### The Vegetative Thallus

A thin electron dense cuticle surrounds the entire vegetative thallus which is 0.1-0.5µm thick. Numerous epiphytes are attached to this covering such as diatoms, Cyanophytes, algae, animals and higher red algae, eg. Ceramium sp. (Fig.38). Between the cuticle and cell wall is a thick mucilage layer, the thallus wall, which is 1.0-1.4µm thick. Each cell is surrounded by its own wall 0.3-0.6µm thick. This cell wall is made up of an inner layer of reticulate fibrils and an outer layer of parallel fibrils. Both layers vary between 0.1 and 0.3µm in thickness. The outer cell wall layer becomes appressed into a dense line between two cells which demarcates the outer limit of each cell. Gaps between cells, analagous to the intercellular space of higher plants, are noticeable and are filled with mucilage (Figs.38 and 39). The plasmalemma, lying between the cell cytoplasm and inner cell wall, is often invaginated or has long extensions into the inner cell wall (Fig.39).

Cells vary in shape from almost square to triangular. Depending on the plane of section and cell age, cell contents appear to be evenly distributed or arranged peripherally around a large central vacuole (Fig.39). Vacuoles are surrounded by a tonoplast as in higher plant and animal cells (Figs.38 and 39). Vacuole contents are electron transparent with floculated ovoid to stellate shaped centres (Fig.38).

Vegetative cells commonly contain more than one ovoid nucleus. The nuclei are surrounded by a double membrane envelope with nuclear pores as in higher plants and animals. Chromatin is often dispersed into dense granular portions and less dense granular areas, the euchromatin and heterochromatin. Usually only one nucleolus is visible (Figs.38 and 39).

Many ovoid chloroplasts are located in each cell, usually peripheral. These are typically florideophycean. Phycobilisomes are present on the thylakoid surfaces. The stroma is rich with ribosomes and occasionally lipid globules are present. DNA fibrils are visible fairly frequently (Fig.38).

The cytoplasm is rich in ribosomes and endoplasmic reticulum which is not always readily visible due to the peripheral nature of the organelles. Mitochondria are usually associated with the nuclei or chloroplasts. Golgi apparatus is not apparent in the vegetative cells. Numerous electron opaque spherical to ovoid floridean starch grains of varying size are scattered in the cytoplasm. Other organelles such as lomasomes, osmiophilic globules and concentric bodies are visible in most cells (Figs.38 and 39).

Pit connections occur between central cells and between central and pericentral cells. These contain a granular plug core usually with flattened surfaces. The mid-region of the pit connection coincides with the cell wall outer limits. The plug cap is present as an extremely electron dense layer covering the plug core surfaces bordering on the cytoplasm. A plug membrane is present although it is often interupted or very convoluted. The plasmalemma is continuous along the sides of the pit connection from cell to cell (Fig.40).

The rhizoids are colourless with rudimentary cell contents restricted to the upper portions nearest the thallus. Most of the rhizoid appears to be made up of vacuoles surrounded by the thin cuticle (Fig.41). The ventral surface of the thallus is not closely appressed to the basiphyte surface and rhizoids do not appear to enter the basiphyte thallus. Epiphytes are abundant on both the basiphyte's and Placophora's ventral surfaces (Fig.42).

## The Reproductive Thallus

### a) The Male Reproductive Structures

The scanning electron micrograph (Fig.43) of the male thallus shows surface morphology but in no greater detail than the light micrographs (Chapter 3). Transmission electron microscopy allowed the entire development from the youngest trichoblast to released spermatium to be seen.

A young trichoblast with a large apical cell and two branch cells with pit connections was the earliest stage seen (Fig.44). These cells contain single central nuclei with large nucleoli and granular chromatin, mitochondria, reduced chloroplasts with one or two thylakoids, a network of rough endoplasmic reticulum with some slightly inflated cisternae, dictyosomes with flattened profiles, a few small vesicles with fibrillar contents associated with both the dictyosomes and the endoplasmic reticulum and ribosome rich cytoplasm (Figs.44 and 45). The trichoblast cells are surrounded by a cell wall, a thick layer of mucilage and a cuticle (Fig.45). The latter consists of an outer layer of thin projections followed by alternating electron dense and electron transparent layers (Fig.46).

Figure 47 shows a later stage of trichoblast development. The polysiphonous condition is now apparent. The extremely thick cell walls, thallus walls and cuticle are constant features. Cell contents of the pericentral cells remain identical to earlier stages although dictyosome activity is now clearly producing small fibrillar vesicles.

Once formed, the pericentral cells act as spermatangial mother cells and cut off several spermatangia (Fig.48). The newly cleaved spermatangia remain attached to the the spermatangial mother cells by ill formed pit connections. Better formed pit connections were not seen in this stage nor in any later stages. Cell organelles are unchanged except that inflated endoplasmic reticulum cisternae are now apparent.

Spermatangial contents become polar with one or two large spermatial vesicles occupying the proximal half and the other organelles the distal half with respect to the position of the spermatangial mother cell (Fig.49). One spermatial vesicle contains a small portion of granular material similar to that within the inflated endoplasmic reticulum cisternae seen earlier (Fig.45). Endoplasmic reticulum and Golgi apparatus with inflated cisternae are visible. A very thin and irregular spermatial wall, which has contents similar in appearance to the Golgi-derived vesicles, has been deposited within the spermatangial wall. Each spermatial vesicle is enclosed by its own thin membrane (Fig.49).

Figure 50 shows a markedly polar mature spermatium within its spermatangium. The spermatial vesicle is proximal and has been excluded from the cytoplasm and the newly laid spermatial wall. Only one spermatial vesicle is present in spermatia at this stage. The spermatial wall consists of an inner layer appressed to the plasmalemma which has parallel fibrils arranged in an amorphous matrix and an outer electron dense layer which has "islands" of reticulate fibrils. The spermatangial wall consists of a very thin layer of reticulate fibrils in an amorphous matrix and an outer, very thin and extremely electron dense membrane which surrounds the released spermatial vesicle and spermatium. Cytoplasmic contents remain unchanged. The thin outer membrane and the cuticle of the spermatangial wall appears to be disintegrating (arrows Fig.50).

A spermatium in the process of being released is shown in figure 51. The two spermatangial wall layers have completely disintegrated. The spermatium together with its two wall layers is emerging to the exterior. The spermatial vesicle remains behind in the spermatangial wall (Fig.52). Scars remain once the spermatial vesicle is reabsorbed (Fig.51). Released spermatia are spherical to ovoid and are still surrounded by their two wall layers (Fig.53).

Figure 54 shows a released spermatium containing the two spermatial wall layers, a plasmalemma, ribosome rich cytoplasm with endoplasmic reticulum, Golgi apparatus, a few small vesicles, mitochondria, plastids and a large central nucleus. The released spermatial contents are very similar to those of early stage spermatia.

### b) The Female Reproductive Structures

Scanning electron micrographs of mature cystocarps show a highly crinkled surface with elongated cells near the ostiole (Fig.55) which is probably a result of preparative techniques since cryo-scanning electron micrographs show these to be smoother with cystocarp cells merely becoming smaller towards the ostiole (Fig.56). This micrograph also shows etched sections through mature cystocarps and trichoblast branches (Fig.56).

Very early developmental stages were not seen. Mature cystocarps were commonly seen with large fusion cells, gonimoblast filaments and carpospores in various stages of development.

The fusion cell (Fig.57) contains a ribosome rich-cytoplasm, mitochondria, a few chloroplasts which appeared degenerate, endoplasmic reticulum and many vacuoles. Gonimoblast filaments were similar in structure to the fusion cell except that fewer vacuoles occurred. Occasionally pit connections were seen between gonimoblast filaments and carpospores. These showed similar morphology to vegetative pit connections but were smaller and the plug membrane was usually degenerating on the gonimoblast filament side (Fig.58).

Fairly well developed carpospores (Fig.59) contain single large nuclei with condensed chromatin and highly invaginated walls, endoplasmic reticulum in a ribosome rich cytoplasm which is associated with the nuclear wall, chloroplasts with a few thylakoids and DNA areas and a few ill-formed floridean starch grains. Golgi apparatus with straight profiles producing a few small vesicles with fibrillar contents, mitochondria associated with Golgi apparatus and the invaginations of the nuclear wall, a few membrane bound bodies of varying size and shape with a striated appearance called striated vesicles, and one or two small areas of fibrillar vesicles which are not clearly membrane bound are also evident. The plasmalemma is invaginated but the cell wall is not well defined.

Figure 60 shows a slightly later stage of development where chloroplasts are fully developed, striated vesicles are well defined, immature starch grains and large membrane bound fibrillar vesicles are present. Dictyosomes (Figs.60 and 61), which are highly curved, have mitochondria at the forming and maturing faces. They are producing vesicles with fibrillar contents some of which have an electron dense core (arrow fig.61).

Mature carpospores (Fig.62) contain a large nucleus with an invaginated nuclear wall and a prominent nucleolus, numerous peripheral chloroplasts, a few fibrillar vesicles, numerous well developed floridean starch grains and dictyosomes with flattened profiles (Fig.62 and 63 arrow) which appear largely inactive or have entire cisternae which are sloughing off (small arrow fig.62). A number of small fibrillar vesicles either with electron dense cores (cored vesicles) or electron dense peripheries (shelled vesicles) are present (Figs.62 and 63). These cored or shelled vesicles are scattered in the cytoplasm and some appear to be fusing with the plasmalemma (large arrow Fig.62). The carpospore wall matures as the spore matures and is shown in figure 64. A young spore wall consists of an inner layer of loosely arranged reticulate fibrils and an outer layer of tightly arranged reticulate fibrils, the two layers being separated by a layer of small electron dense globules (small arrows Fig.64). A mature spore wall contains these two layers and a further new inner layer, adjacent to the plasmalemma, having a swirled appearance and made up of loosely arranged fibrils. The mucilage of the cystocarp is appressed between the two carpospore walls and appears as a layer of electron dense globules in an amorphous matrix (large arrows Fig.64). The plasmalemma of the mature spore is highly invaginated while that of the young spore is not so marked.

Figure 65 is a cryo-scanning micrograph of a mature cystocarp showing the pericarp, gonimoblast filaments, carpospores in various develpomental stages and the fusion cell.

Released carpospores are similar in appearance to mature spores (Fig.66) although chloroplasts are no longer peripheral. A few starch grains are apparent, fibrillar vesicles are small and ill formed, cored and shelled vesicles are still numerous and the carpospore wall is ill defined. Cored vesicles still appear to be fusing with the plasmalemma. Dictyosomes are present but few in number and have straight profiles and no vesicles (inset Fig.66). Mitochondria are present and occur near the plasmalemma (Figs.67 and 68). Cored vesicles appear to empty their contents to the exterior (Fig.67) by fusing with the plasmalemma. The external wall is loosely fibrillar (Fig.67). Small microtubule-like structures occur near the plasmalemma and are associated with electron dense globules almost as if the globule had been extruded via the tubule (Fig.68).

Scanning electron micrographs of released carpospores (Fig.69) show irregular surfaces and indented pores. Mucilage is still apparent (arrow Fig.69) but has mostly been removed by the preparative process. Figure 70 shows a carpospore in an early stage of germination. Pores are no longer visible.

### c) The Tetrasporic Structures

Due to the sequential development of tetrasporangia within in the tetrasporangial branch (chapter 3) it was possible to view more developmental stages than in the female. Both carpospores and tetraspores are very similar in their ultrastructure and only marked differences or outstanding features of the tetraspores will be discussed below.

Figure 71 shows a newly developed tetrasporangium. Cleavage furrows occur and appear as invaginations of the plasmalemma. Nuclear division has occurred and here two nuclei are visible with invaginated walls, condensed chromatin and single nucleoli. Depending on the plane of section two or three, nuclei were visible. Many mitochondria, chloroplasts with a few thylakoids and immature floridean starch grains are present. Endoplasmic reticulum in a ribosome rich cytoplasm also occurs. A few granular to slightly striated vesicles are associated with endoplasmic reticulum and possibly early stage dictyosomes (Figs.72 and 73).

A young tetrasporangium (Fig.74) contains large fibrillar vesicles, striated vesicles, dictyosomes with curved profiles, mature starch grains and chloroplasts. It has a general appearance similar to carpospores (Fig.60) at the same stage. A pit connection occurs between the fertile pericentral cell and the tetrasporangium (Fig.74). Cleavage furrows have deepened.

Figure 75 shows a nearly mature tetrasporangium where cleavage furrows are nearly complete. Spore contents and wall composition are identical to that of a mature carpospore (Fig.62) except that here two to three nuclei are visible depending on the plane of section.

Released tetraspores are also identical to carpospores in every way. Figure 76 shows a released tetraspore nucleus which has a smooth wall and a single nucleolus. A scanning electron micrograph of a released tetraspore (Fig.77) also shows the marked similarity between the carpospores and tetraspores.

## 4.3 DISCUSSION

### The Vegetative Thallus

The cell wall comprises a cuticle, mucilage layers and a plasmalemma. The "hint of a middle lamella" seen by Kugrens and West (1973) in Choreocolax polysiphoniae corresponds to the appressed dense line between the outer cell walls of two adjacent cells. The cuticle probably functions as a protective layer against epiphyte intrusion, desiccation or the loss of the underlying mucilage wall as indicated by Bisalputra et al (1967) in Laurencia spectabilis. The often irregular appearance of the plasmalemma and the occurrence of plasmalemmavillae (Brown and Weier 1970) appear to confirm the popular theory of wall material being secreted through the plasmalemma. However this work supports the speculation that the exact appearance of the wall layers is dependent on fixation procedures (Duckett and Peel 1978) as the plasmalemmavillae are not always present and usually only in areas where fixation appears to have been inadequate.

The extreme vacuolation of older cells may confirm the hypothesis of Bisalputra et al (1967) that the cell wall is made up of cytoplasmic contents, which are removed through the plasmalemma, leaving the cell with a thin peripheral cytoplasmic layer and a large central vacuole.

The structure of the chloroplast, vacuole, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, floridean starch grains and ribosome rich cytoplasm are all typical of most Florideophycideae.

Pit connection structure in the vegetative cells confirms that described by Pueschel and Cole (1982) for Rhodomelaceae in that no cap layers occur in Placophora binderi. The role of the plug membrane in nutrient transport as indicated by its presence or absence (Wetherbee 1979; Wetherbee and Scott 1980; Quirk and Wetherbee 1980) is doubtful, unless proof other than transmission electron microscopy for its existence can be supplied as the visualisation of the exact structure in any species is dependent on fixation procedure and the thickness of the plug cap (Pueschel 1977). The continuity of the plasmalemma along the sides of the pit connection may be indicative of cytoplasmic continuity as the pit connection may then be regarded as intracytoplasmic (Bisulputra et al 1967; Bouck 1962; Ramus 1969b). This report does not confirm or refute the debate on function. However it appears that the formation of such a structure, usually between all cells within a thallus, supports the theory that they maintain some form of cytoplasmic continuity, not necessarily that of nutrient transport (Ramm-Anderson and Wetherbee 1982).

The lack of any substantial organs in the rhizoids appears to confirm that their function is primarily anchorage (chapter 3).

## The Reproductive Thallus

### a) The Male Structures

The typical feature of spermatial development, the spermatial vesicle, is present in Placophora binderi. It appears to be derived by the coalescence of endoplasmic reticulum cisternae during early development as in Polysiphonia hendryi (Kugrens 1980) and Levringiella gardneri (Kugrens and West 1972b) and not dictyosome derived as in Ptilota densa (Scott and Dixon 1973b), Griffithisia flosculosa (Peyrière 1971) and Erythrocystis saccata (Kugrens and West 1972b). It may however be added to by dictyosome derived vesicles during later development as in Janczewskia gardneri (Kugrens 1974) and Tiffaniella snyderae (Fetter and Neushul 1981). This is indicated by the presence of vesicles comprising a loosely fibrillar component reminiscent of Golgi derived vesicles during the latter stage of the formation of the spermatia. It is possible that this is the case for all species which produce spermatial vesicles, but studies incorporating all developmental stages would be needed to confirm this.

It is generally conceded that the spermatial wall is dictyosome derived which appears to be true for Placophora binderi. As a distinct wall forms around each spermatium within the spermatangial wall, the latter remaining behind together with the spermatial vesicle as in Ptilota densa (Scott and Dixon 1973b) and in

Janczewskia gardneri (Kugrens 1974), the classical terminology as applied by Scagel (1953) holds for Placophora binderi.

The method of spermatium release appears to follow that described for Polysiphonia hendryi (Kugrens 1980). The spermatial vesicles fuse and become extracytoplasmic as described by Fetter and Neushul (1981), Scott and Dixon (1973b), Peyrière (1971,1974), Kugrens (1980), Kugrens and West (1972b) and Peel and Duckett (1975). The general enlargement of spermatangia with increasing spermatial vesicle size causes a general increase in pressure within the spermatangial branch. This results in older spermatangia being forced towards the surface. The cuticle of the spermatangial branch appears to peel away as in Chondrus crispus (Tveter-Galagher et al 1980). The spermatangial wall ruptures, allowing the spermatia to be forcefully released as indicated by the presence of scars in the spermatangial branch. The spermatial vesicle is left behind once the spermatium has been released and the spermatial wall reforms. The spermatial vesicle in this case appears to function primarily in release of the spermatium and not as an adhesive.

### b) The Female and Tetrasporic Structures

Carpospore and tetraspore development in Placophora binderi follow identical patterns similar to that described for most species, especially members of the Ceramiales (see chapter 1). The irregular nuclear membrane seen in all stages appears to agree with

Pueschel's (1979) hypothesis that it is actively involved in spore formation during the early stages of development as few organelles occur near the nucleus to cause pressure. In later stages organelles may create pressure on the nuclear membrane when their number is greatly increased. The smooth nuclear membrane seen in released spores, even though organelle number is still high, lends further support to Pueschel's (1979) theory.

Three vesicle types are produced in carpospores and tetraspores of Placophora binderi: striated vesicles, fibrillar vesicles and cored vesicles. The striated vesicles are reminiscent of those described by most authors and of the paracrystalline inclusions described in Neurospora crassa (Wood and Luck 1971) and the crystalline bodies of Antithamnion (Young and West 1979). Fritsch (1935), using light microscopy, described vesicles of this nature as a feature of Ceramiales spores using light microscopy. They appear to be formed early in development by endoplasmic reticulum as seen by the association of endoplasmic reticulum with finely granular and later slightly striated microbodies in early stage spores. An endoplasmic reticulum origin is generally proposed by most authors. A protein structure has been generally claimed from both cytochemical tests and the structural similarity of striated vesicles to protein bodies in other species. The commonly proposed function of a protein store for later development appears to be reasonable as these vesicles are transient, appearing and disappearing within the first few early stages of spore development.

The fibrillar vesicles in Placophora binderi are probably dictyosome derived during early sporogenesis, as described in Caloglossa leprieuri (Triemer and Vasconcelos 1977), Polysiphonia sertularioides (Tripodi 1971), Polysiphonia novae-angliae (Wetherbee and West 1977) and Ptilota hypnoides (Scott and Dixon 1973a). Some authors propose an early endoplasmic reticulum origin, followed by later dictyosome involvement (Alley and Scott 1977; Kugrens and West 1973a,1974; Pueschel 1979). Fibrillar vesicles only appear as recognisable structures together with increasing Golgi activity soon after sporogenesis commences thus a dictyosome origin is indicated. Fibrillar vesicles remain apparent in released spores although their boundaries are ill defined, indicating that they may be acting as a carbohydrate store. This is a commonly attributed function. Another proposed function, that of spore wall deposition does not appear to operate in Placophora binderi. This appears to be more the responsibility of the small dictyosome derived vesicles which form during fibrillar vesicle enlargement and which appear to fuse with either the fibrillar vesicle or the wall.

The cored vesicles start appearing just prior to spore maturity, becoming the most numerous organelle in spores at release. The shelled vesicles probably represent a shift in position of the dark material as in Levringiella gardneri (Kugrens and West 1972c). In Placophora binderi the cored vesicles are most likely to be dictyosome derived. Just prior to maturity they may form by dictyosome cisternae wrapping around cytoplasm to produce cored vesicles and later by entire cisternae sloughing off as described in Polysiphonia (Hawkins 1974a), Callithamnion roseum (Hawkins 1974b), Nemalion helminthoides (Ramm-Anderson and Wetherbee 1982) and Ptilota hypnoides (Scott and Dixon 1973a). Their function in Placophora binderi appears to be primarily that of an adhesive once spores start germination as in Ceramium rubrum (Chamberlain and Evans 1973) and Polysiphonia (Wetherbee 1978). The cored vesicles are still numerous in newly settled spores and do not appear to play an active role in wall deposition as is claimed for other species. The spore wall may provide a primary tenuous adhesive at initial spore settlement, which is later intensified by the secretion of the cored vesicles as in Ceramium rubrum (Chamberlain and Evans 1973). The core appears to be released first as described by Wetherbee (1978). The release of these vesicles may leave a "scar" in the form of small microtubule-like structures with the dense globule being the remains of the core or fibrillar material. This scar may, however, also represent microorganism infection sites as the spores are susceptible to this in culture.

The remarkable similarity of ultrastructural features in carpospore and tetraspore development is to be expected as both spores produce entire new adult generations and have to go through similar processes even though they have different ploidy levels.

Fig. 38: Section through a mature vegetative thallus (TEM).

LEGEND:

b- concentric body
c- chloroplast
ch- chromatin
cu- cuticle
cw- cell wall
D- DNA fibrils
ep- epiphytes
fs- floridean starch grain
g- cell gap
i- inner layer
l- lipid droplet
lo- lomosome
N- nucleus
o- outer layer
og- osmiophilic globule
p- pit connection
tw- thallus wall
v- vacuole

38

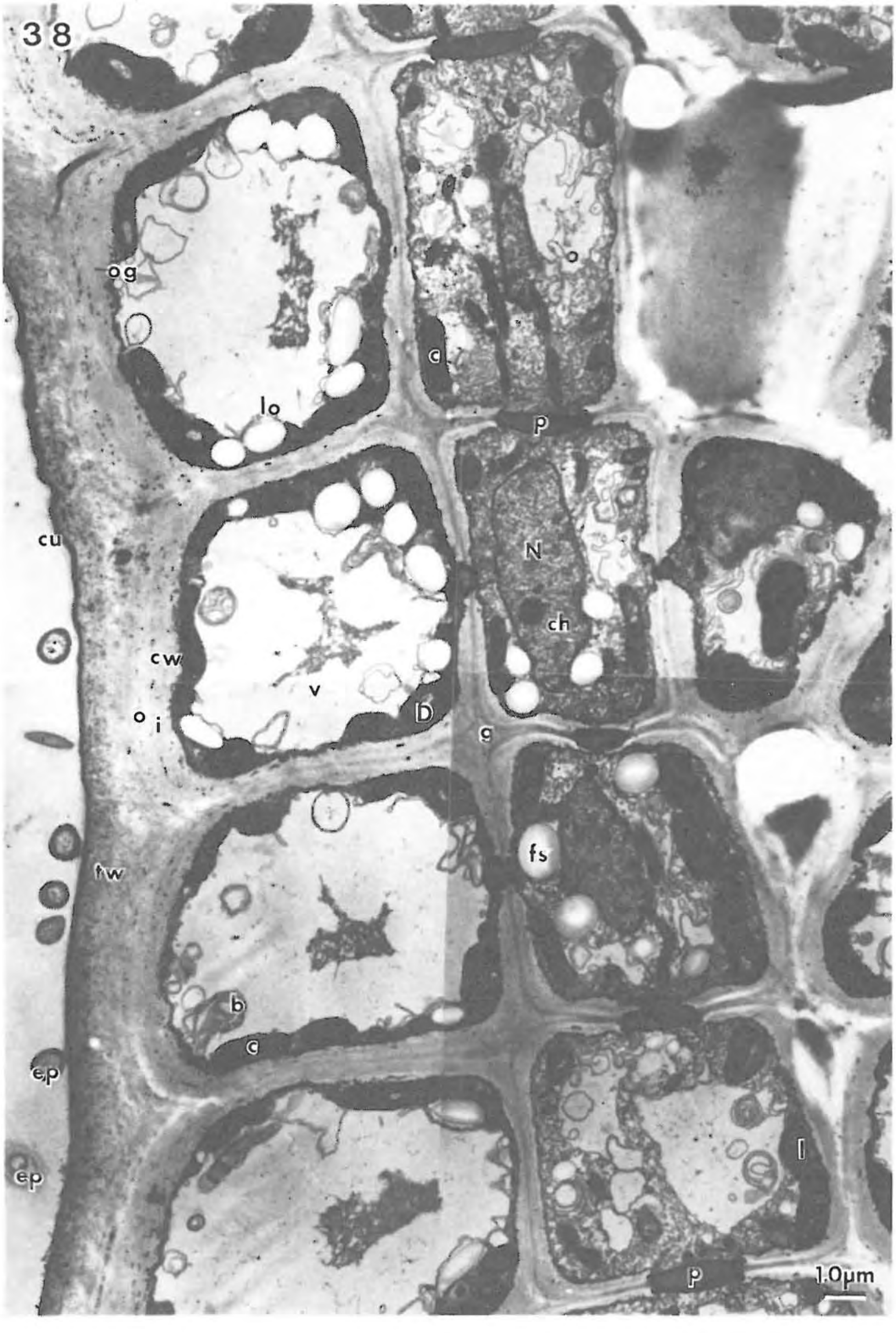
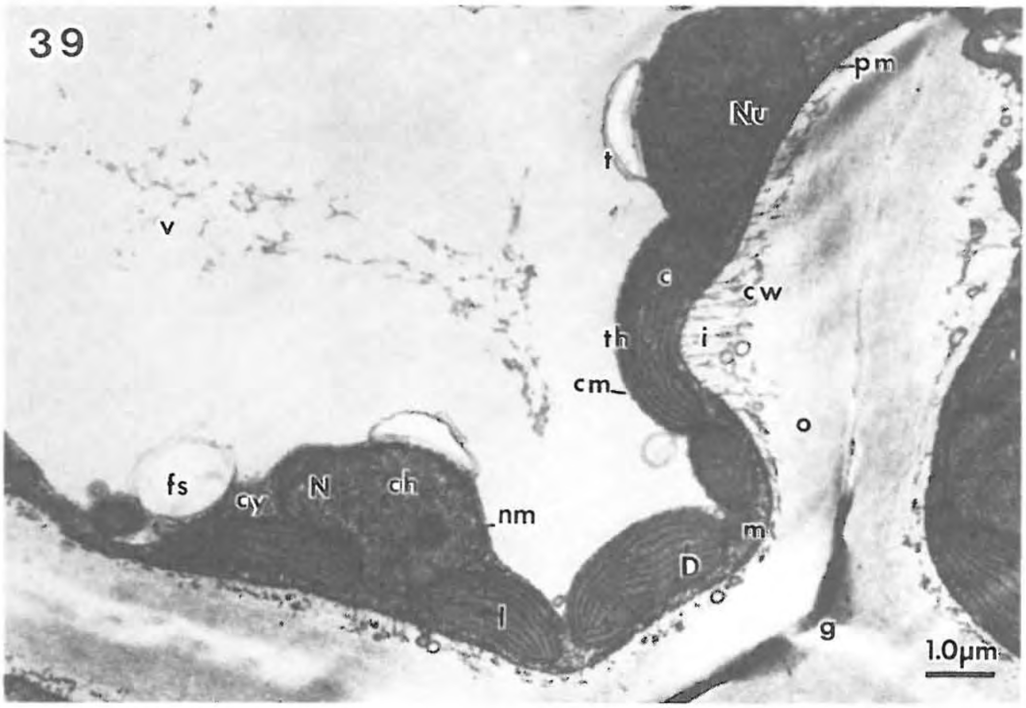


Fig. 39: Mature vegetative cell (TEM).

Fig. 40: Pit connection between two central cells of a mature vegetative thallus (TEM).

LEGEND:

c- chloroplast
cc- central cell
ch- chromatin
cm- chloroplast membrane
cw- cell wall
cy- cytoplasm
D- DNA fibrils
fs- floridean starch grain
g- cell gap
i- inner layer
l- lipid droplet
m- mitochondrion
N- nucleus
nm- nuclear membrane
Nu- nucleolus
o- outer layer
pa- plug cap
pe- plug membrane
pl- plug core
pm- plasmalemma
t- tonoplast
th- thylakoid
v- vacuole


39
pm
Nu
t
v
c
cw
th
i
cm
o
fs
cy
N
ch
nm
m
D
l
g
1.0µm

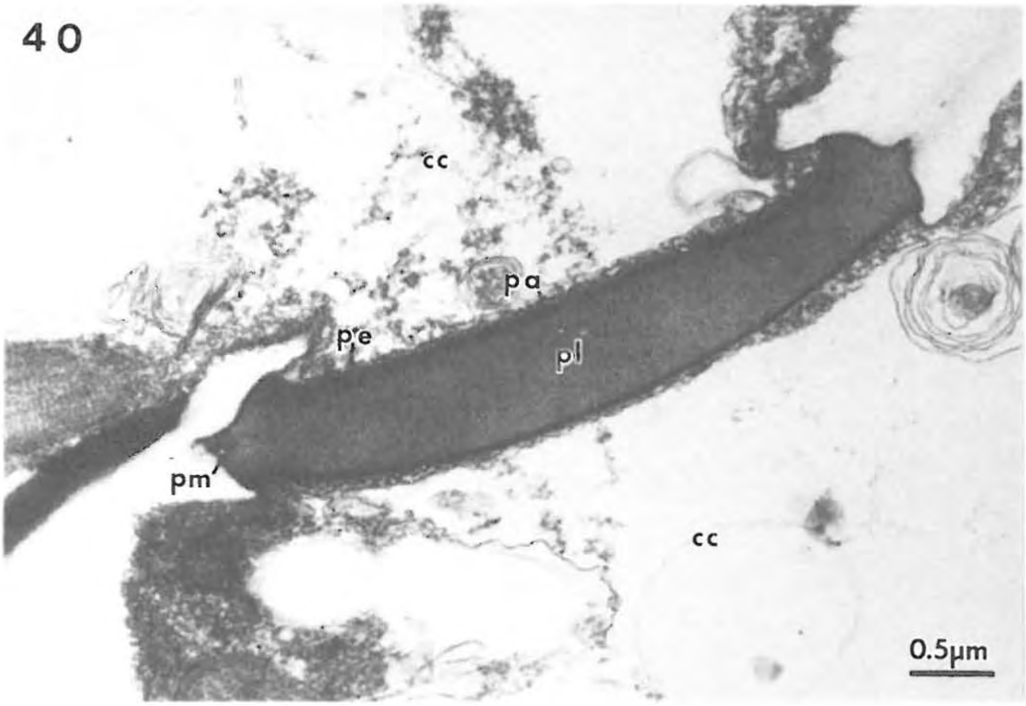
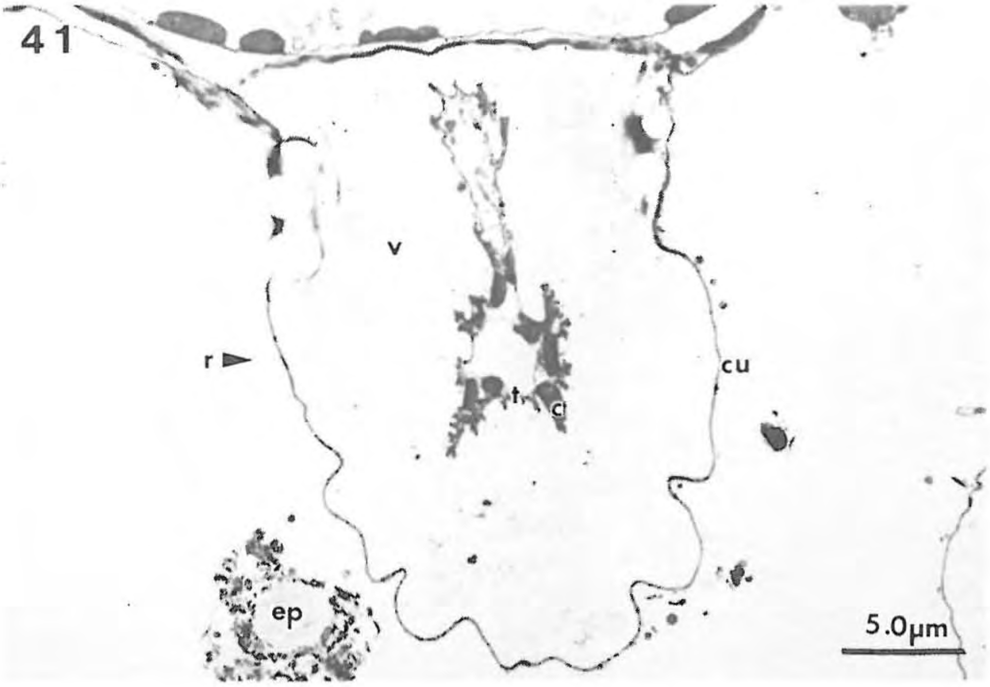

40
cc
pa
pe
pl
pm'
cc
0.5µm

Fig. 41: Longitudinal section of a rhizoid from a mature thallus (TEM).

Fig. 42: Rhizoids coming off the ventral surface of the thallus and lying between Codium (the basiphyte) utricles (SEM).

LEGEND:

B- basiphyte
c- chloroplast
cu- cuticle
ep- epiphytes
Pb- Placophora binderi
r- rhizoid
t- tonoplast
v- vacuole


41

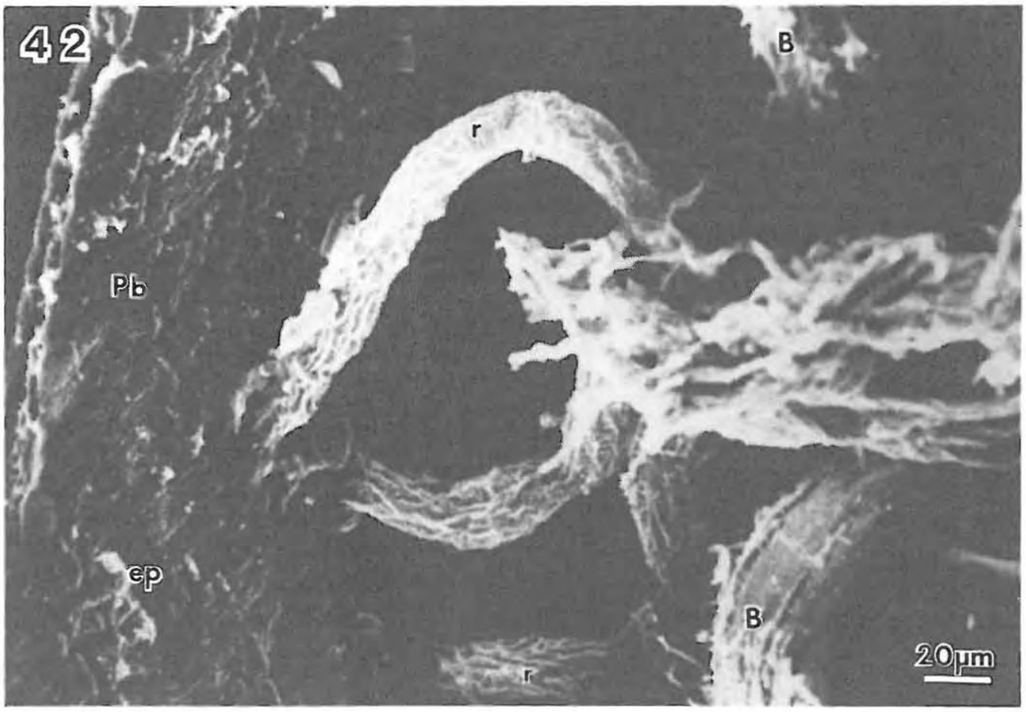

42

Fig. 43: Mature spermatangial branches (TEM).

Fig. 44: Section through a young trichoblast from a male gametophyte (TEM).

LEGEND:

c- chloroplast

ch- chromatin

cu- cuticle

cw- cell wall

er- endoplasmic reticulum

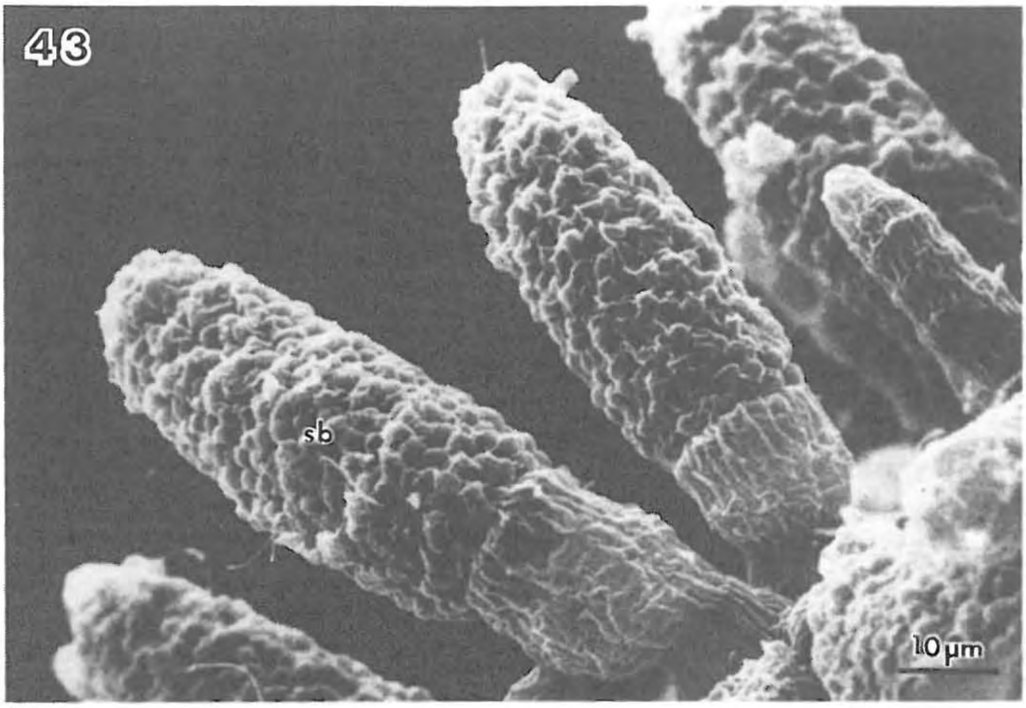
mu- mucilage

N- nucleus

Nu- nucleolus

p- pit connection

sb- spermatangial branch


43
sb
10 µm

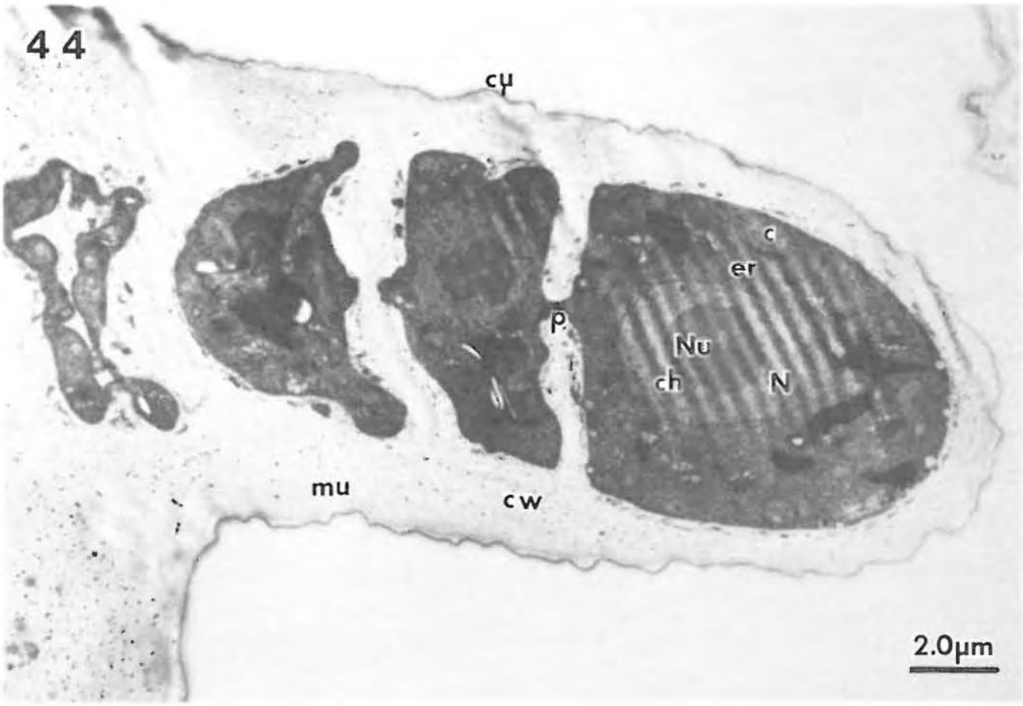

44
cu
c
er
p
Nu
ch
N
mu
cw
2.0µm

Fig. 45: Apical cell from a young trichoblast on a male gametophyte (TEM).

Fig. 46: Section of the cuticle and wall of a trichoblast on a male gametophyte (TEM).

LEGEND:

c- chloroplast

ch- chromatin

cu- cuticle

cw- cell wall

d- dictyosome

ed- electron dense layer

er- endoplasmic reticulum

et- electron transparent layer

m- mitochondrion

mu- mucilage

N- nucleus

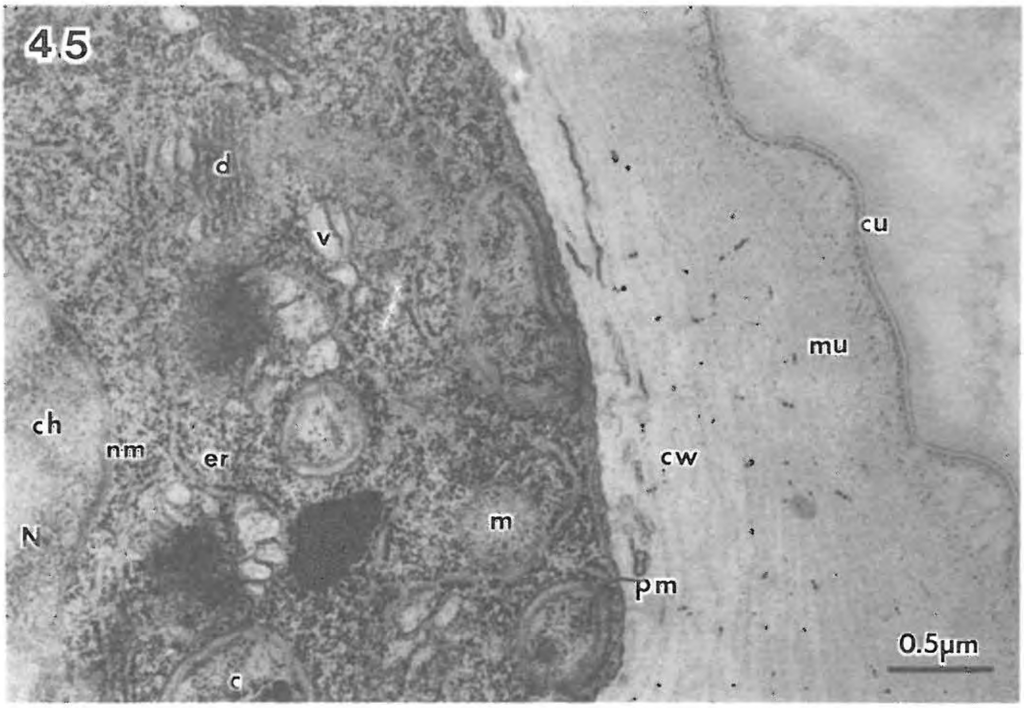
nm- nuclear membrane

pm- plasmalemma

v- vacuole

*- thin projections

45



46

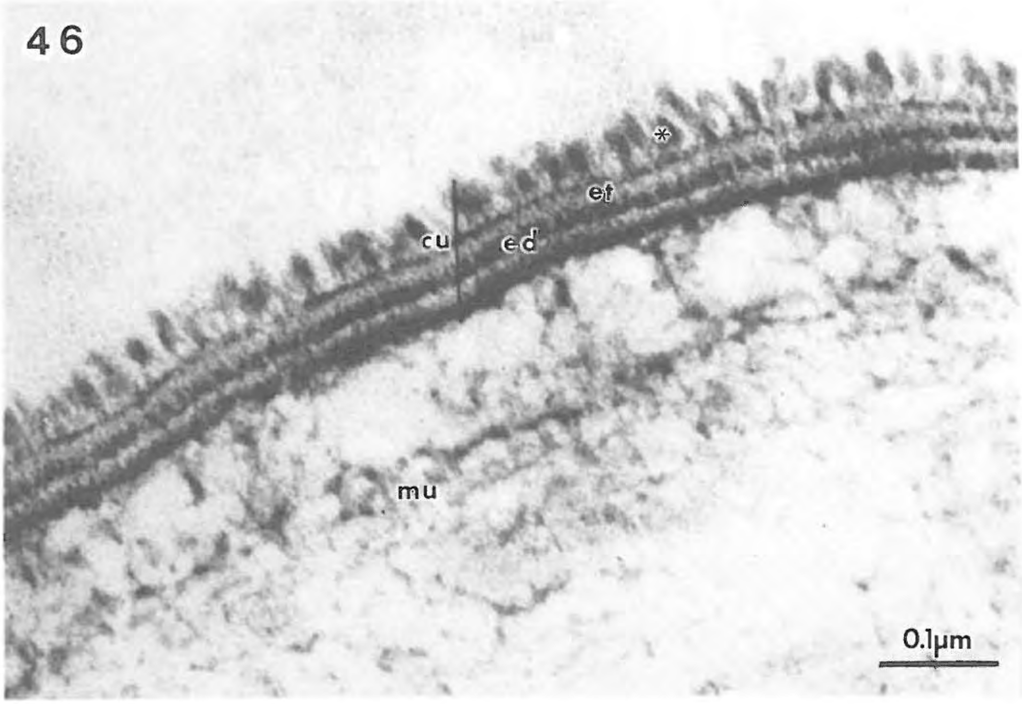


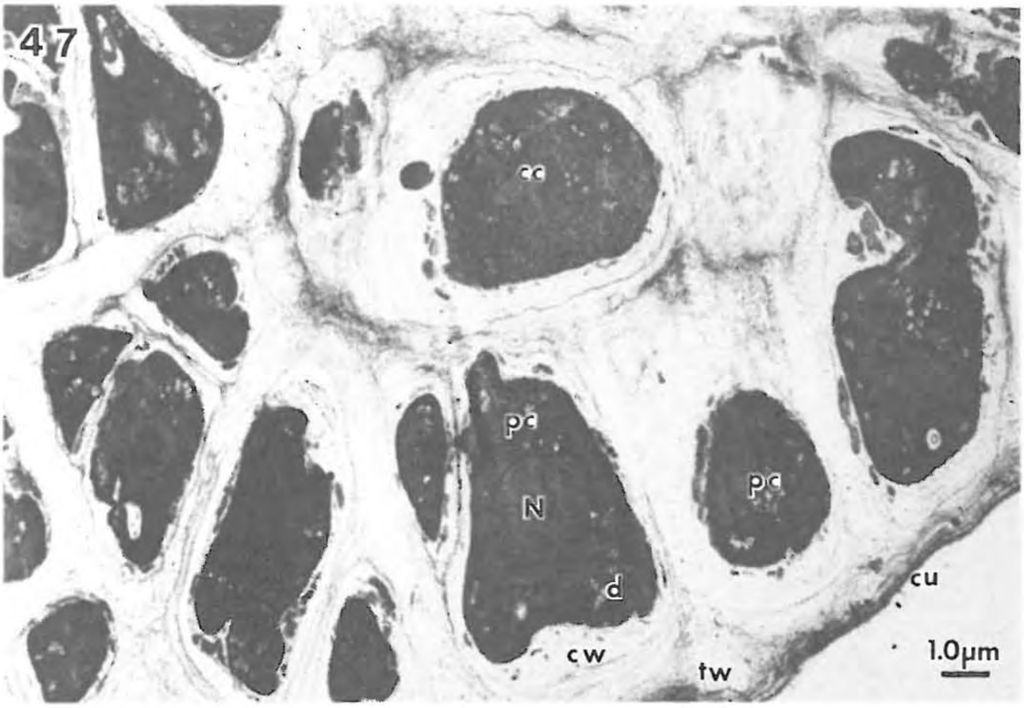
Fig. 47: Trichoblast becoming polysiphonous on a male gametophyte (TEM).

Fig. 48: Young spermatangial mother cell cutting off spermatangia (TEM).

LEGEND:

cc- central cell
cu- cuticle
cw- cell wall
d- dictyosome
er- endoplasmic reticulum
N- nucleus
p- pit connection
pc- pericentral cell
sm- spermatangium
smc- spermatangial mother cell
tw- thallus wall

47



48

Fig. 49: Young spermatangia (TEM).

Fig. 50: Mature spermatangium (TEM).

LEGEND:

cu- cuticle

d- dictyosome

er- endoplasmic reticulum

fs- floridean starch grain

gm- granular material

i- inner layer

mu- mucilage

N- nucleus

o- outer layer

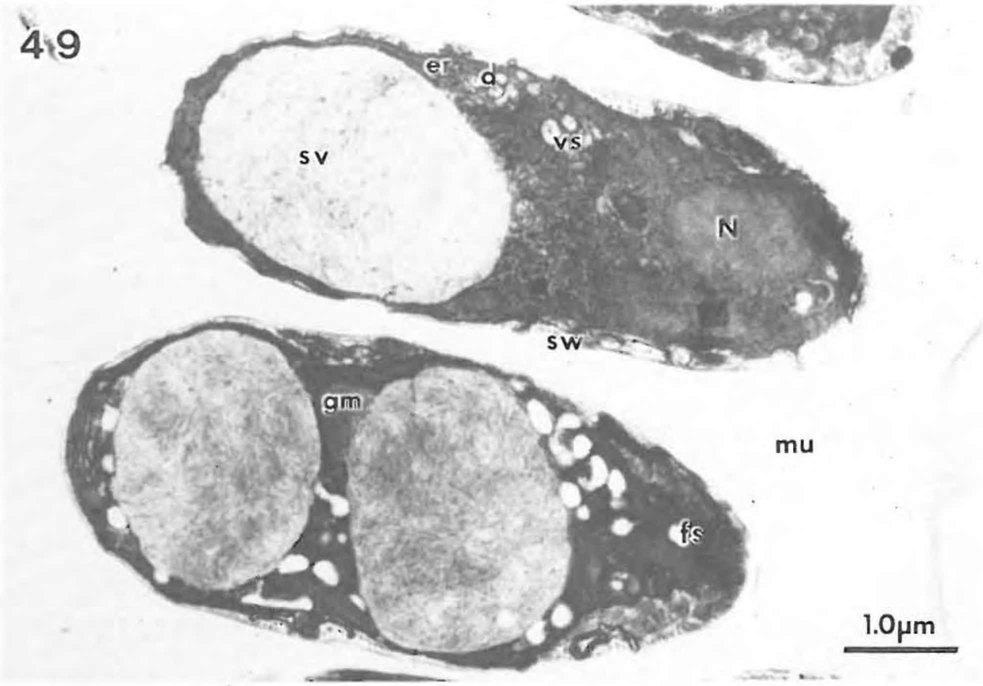
q- inner layer of the spermatangial wall

qo- outer spermatangial membrane

sv- spermatial vesicle

sw- spermatial wall

vs- vesicle


49
er
d
sv
vs
N
sw
gm
mu
fs
1.0μm

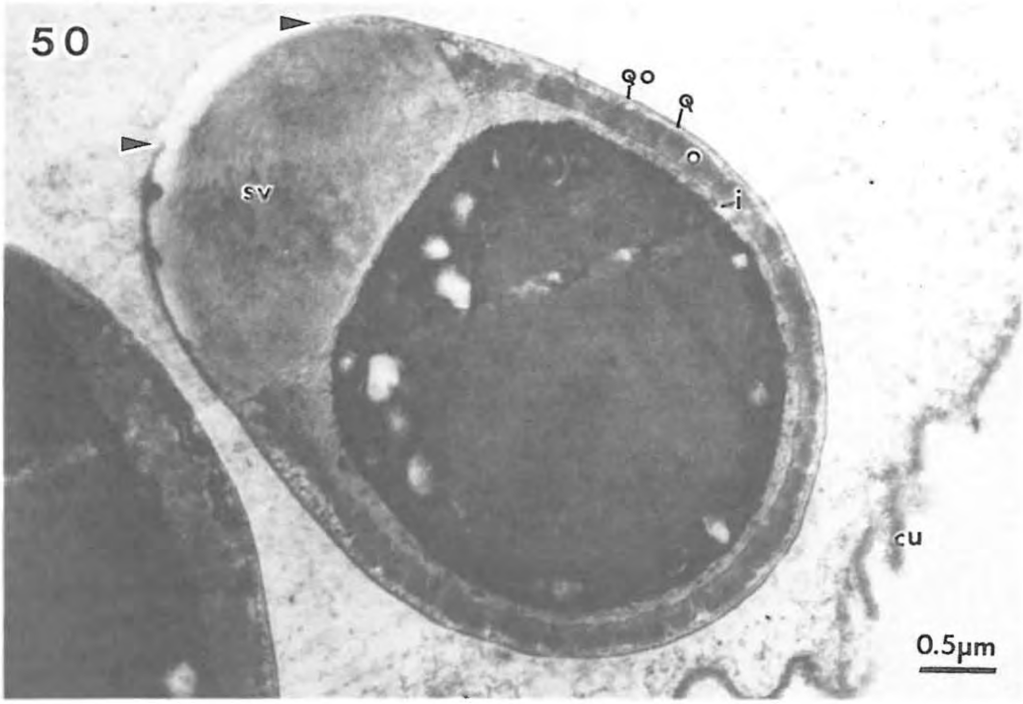
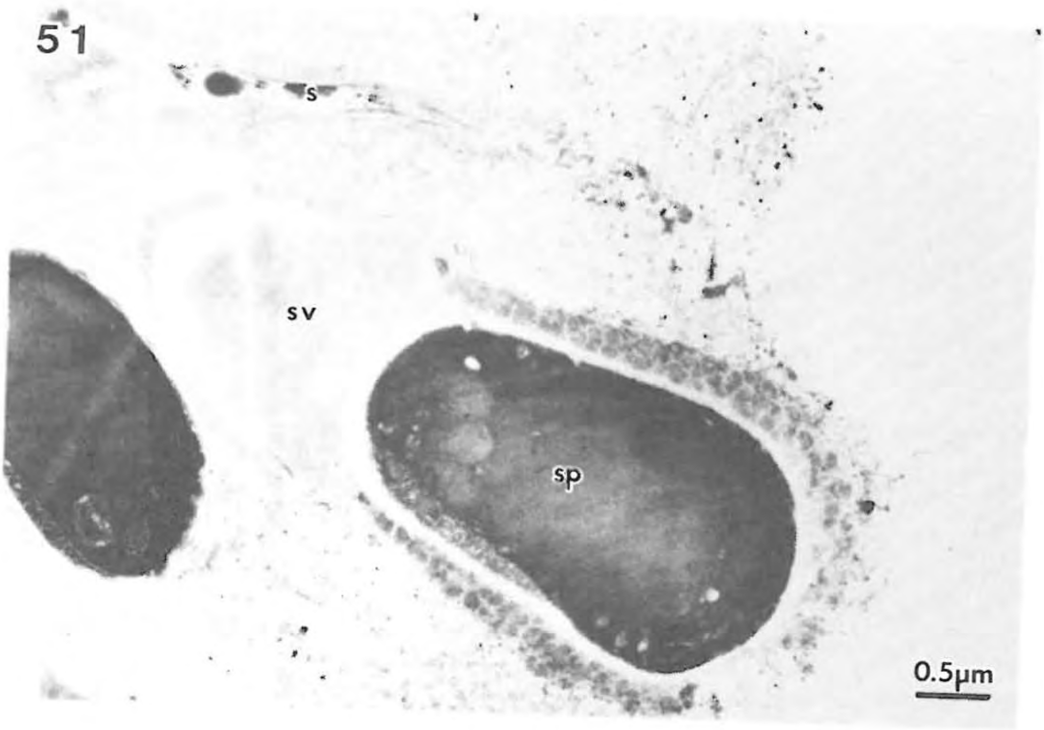

50
o
i
o
i
o
i
sv
cu
0.5μm

Fig. 51: Spermatium being released (TEM).

Fig. 52: Newly released spermatium (TEM).

LEGEND:

c- chloroplast
cu- cuticle
d- dictyosome
fs- floridean starch grain
i- inner layer
N- nucleus
o- outer layer
sp- spermatium
sv- spermatial vesicle
vs- vesicle
s - scar


51
s
sv
sp
0.5µm

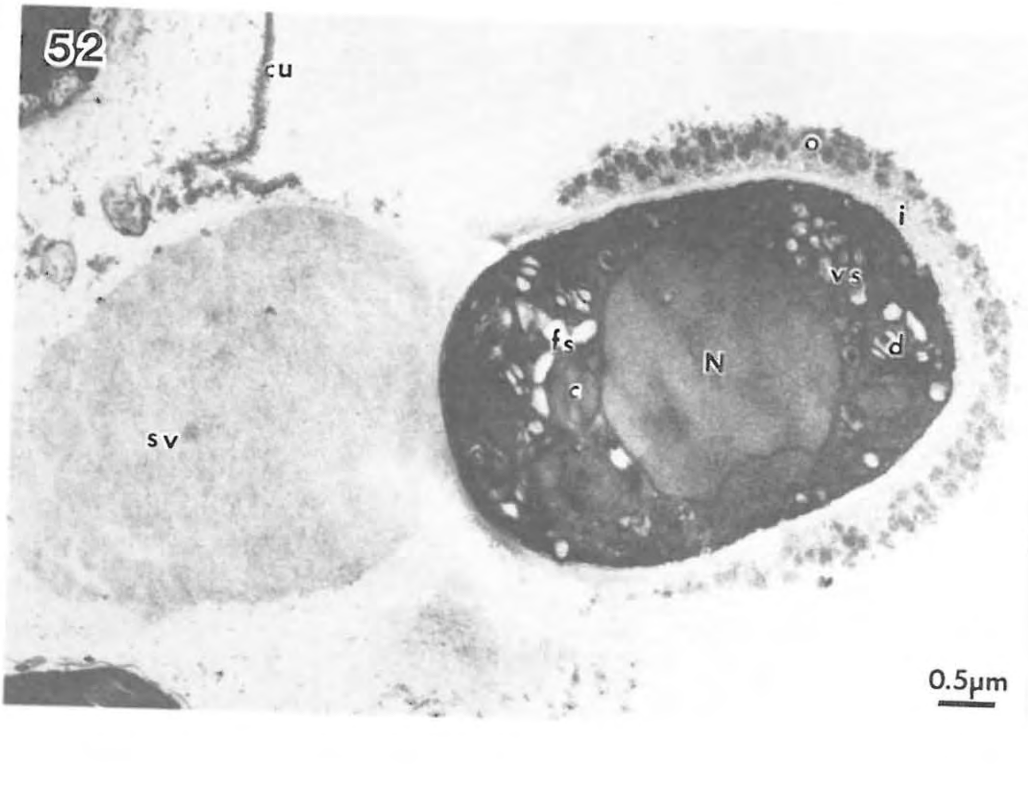

52
cu
o
i
vs
fs
d
N
c
sv
0.5µm

Fig. 53: Released spermatium (TEM).

Fig. 54: Released spermatium (TEM).

LEGEND

c- chloroplast

cy- cytoplasm

d- dictyosome

er- endoplasmic reticulum

fs- floridean starch grain

N- nucleus

nm- nuclear membrane

pm- plasmalemma

sp- spermatium

sw- spermatial wall

53

54

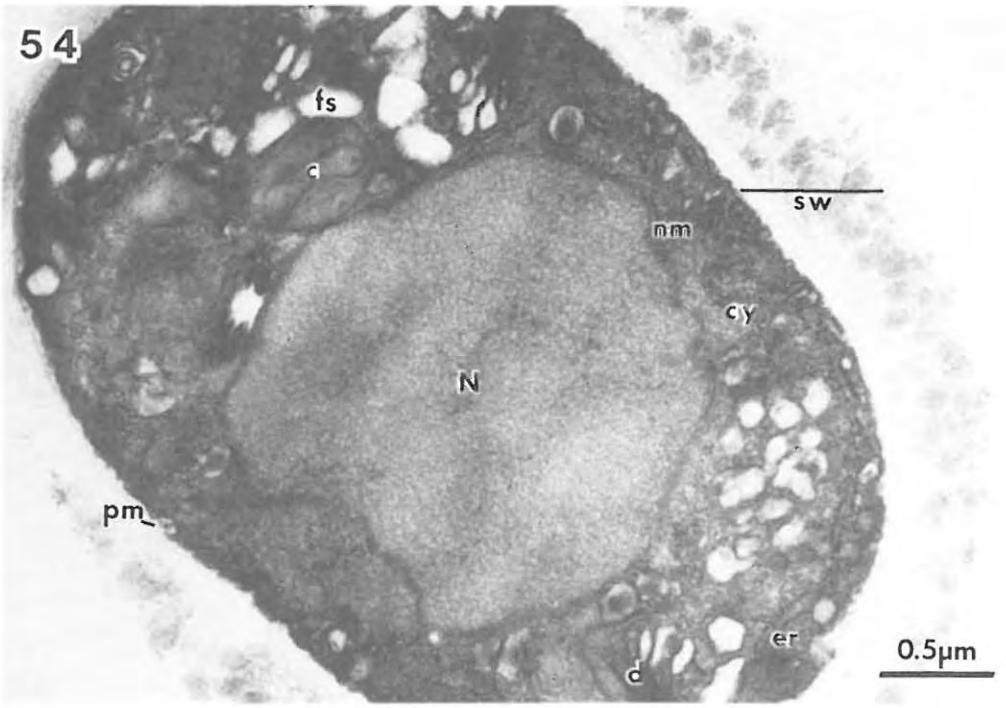


Fig. 55: Mature cystocarp on a female thallus (SEM).

Fig. 56: Group of mature cystocarps on a female thallus (Cryo-SEM).

LEGEND

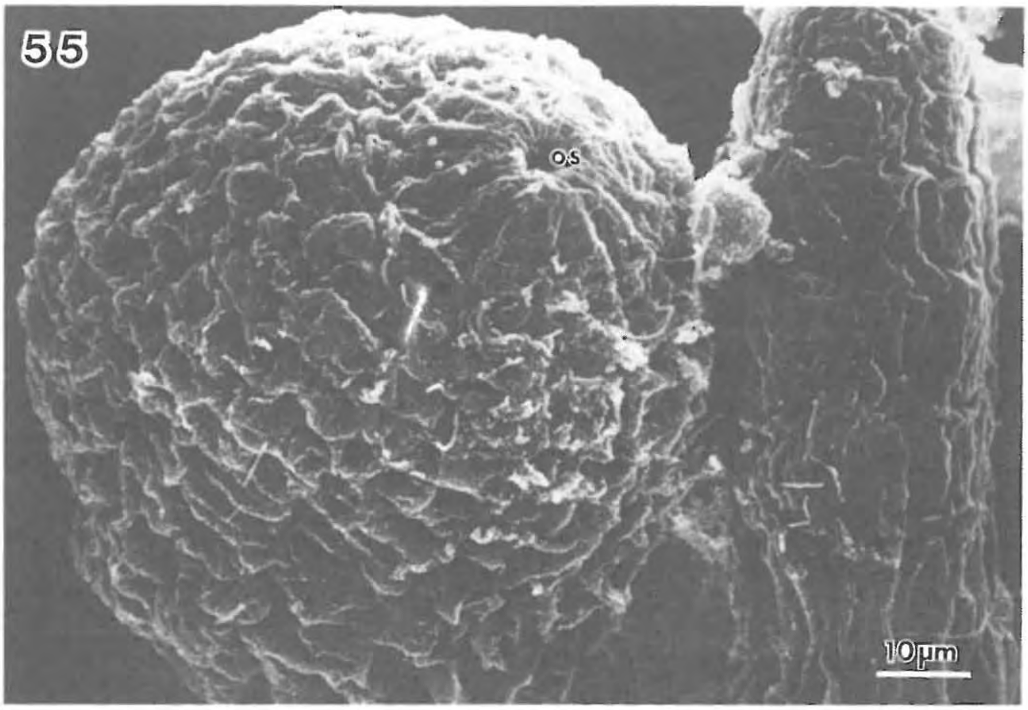
cs- carpospore

cy- cystocarp

E- erect reproductive branch

f- fusion cell

os- ostiole


55
o.s
10µm

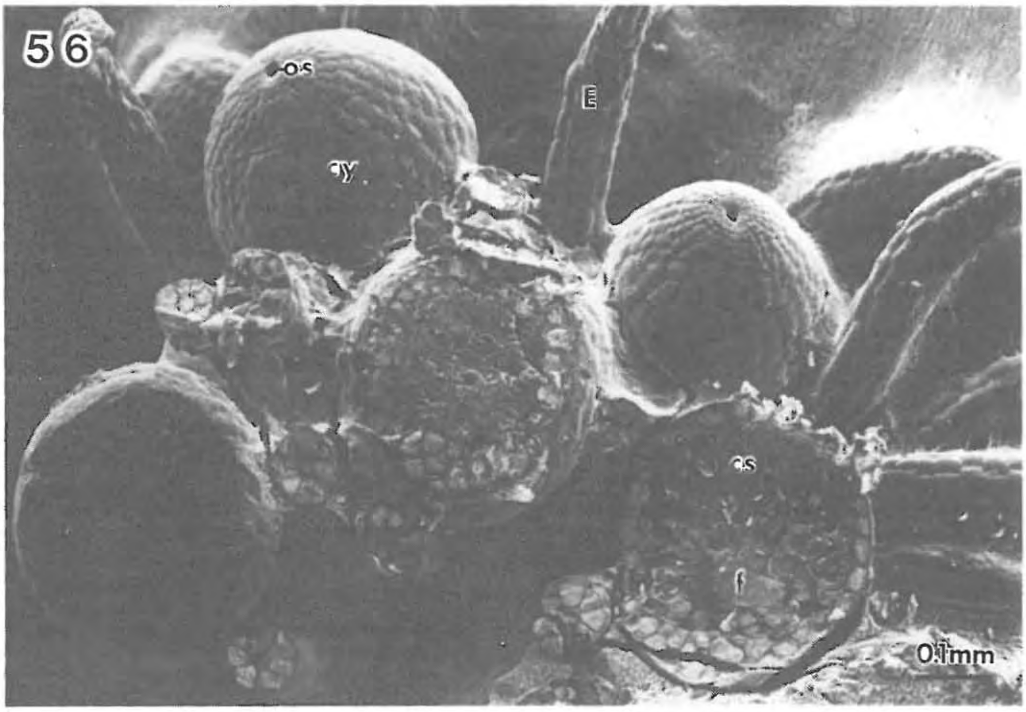
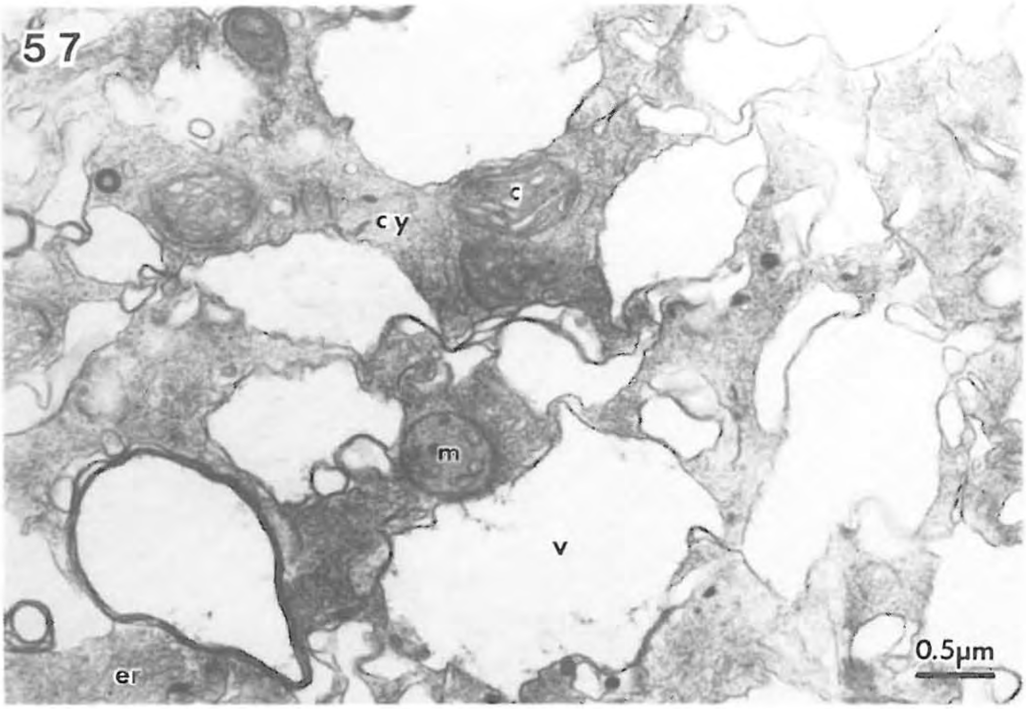

56
o.s
E
cy
cs
f
0.1mm

Fig. 57: Fusion cell of a mature cystocarp (TEM).

Fig. 58: Pit connection between a gonimoblast filament and a young carpospore (TEM).

LEGEND:

c- chloroplast
cs- carpospore
cy- cytoplasm
er- endoplasmic reticulum
go- gonimoblast cell
m- mitochondrion
p- pit connection
v- vacuole


57
c
cy
m
v
er
0.5µm

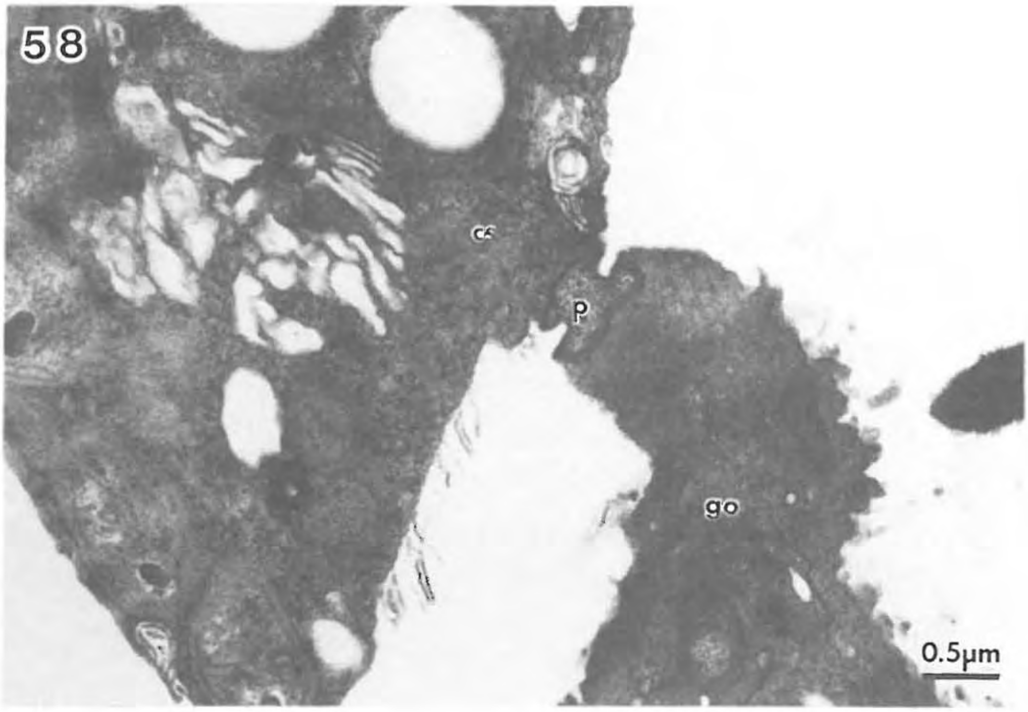

58
cs
p
go
0.5µm

Fig. 59: Newly developed carpospore (TEM).

Fig. 60: Young carpospore (TEM).

LEGEND:

c- chloroplast

ch- chromatin

d- dictyosome

er- endoplasmic reticulum

ff- forming face of dictyosome

fm- maturing face of dictyosome

fs- floridean starch grain

fv- fibrillar vesicle

m- mitochondrion

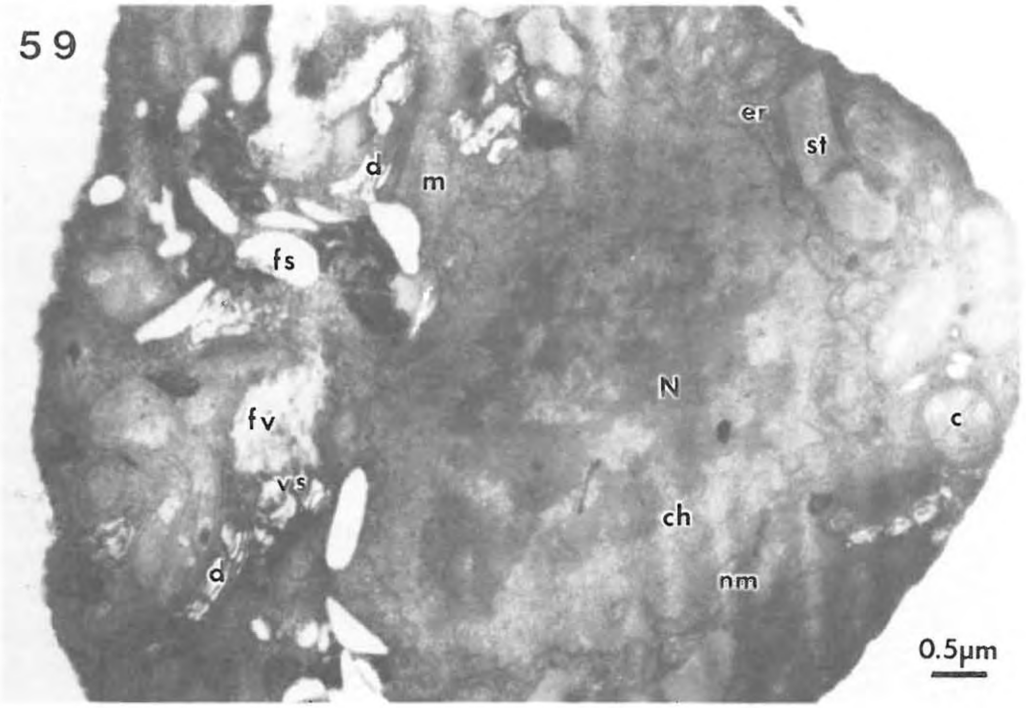
N- nucleus

nm- nuclear membrane

st- striated vesicle

vs- vesicle

59



60

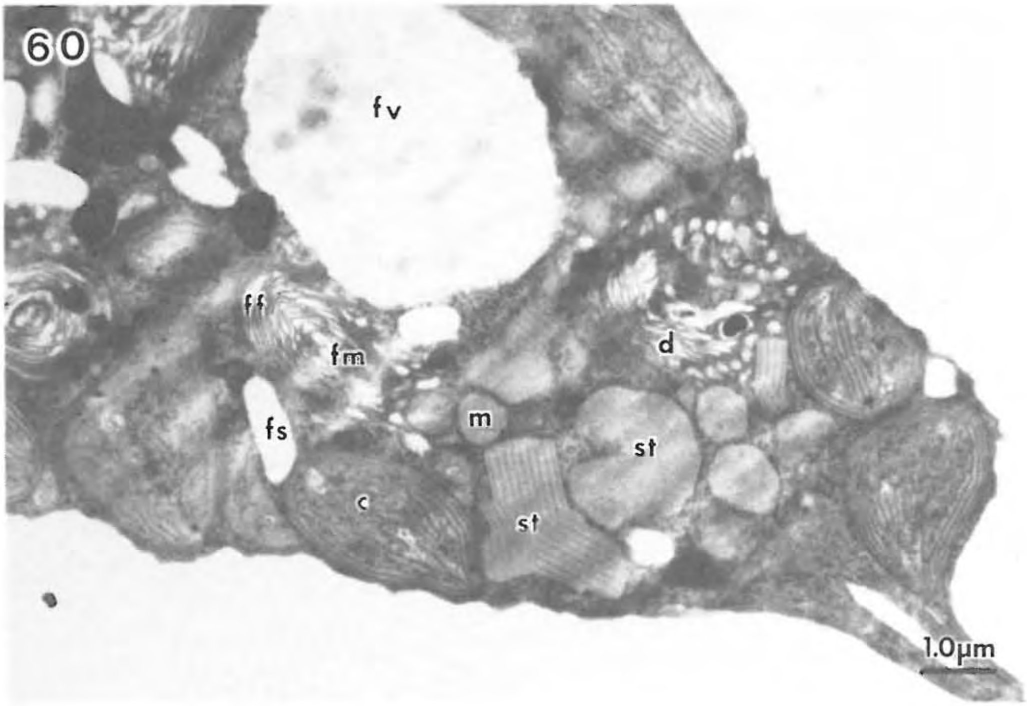
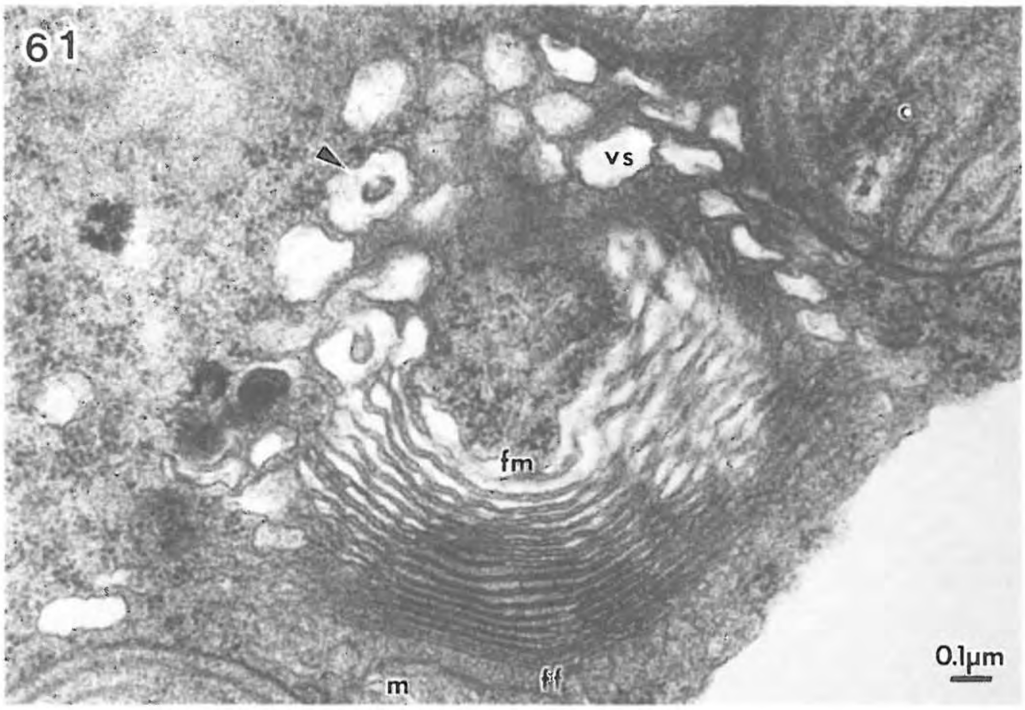


Fig. 61: Dictyosome in a young carpospore (TEM).

Fig. 62: Mature carpospore (TEM).

LEGEND:

c- chloroplast
cv- cored vesicle
ff- forming face of dictyosome
fm- maturing face of dictyosome
fs- floridean starch grain
fv- fibrillar vesicle
m- mitochondrion
N- nucleus
Nu- nucleolus
sh- shelled vesicle
vs- vesicle


61
c
vs
fm
m
ff
0.1µm

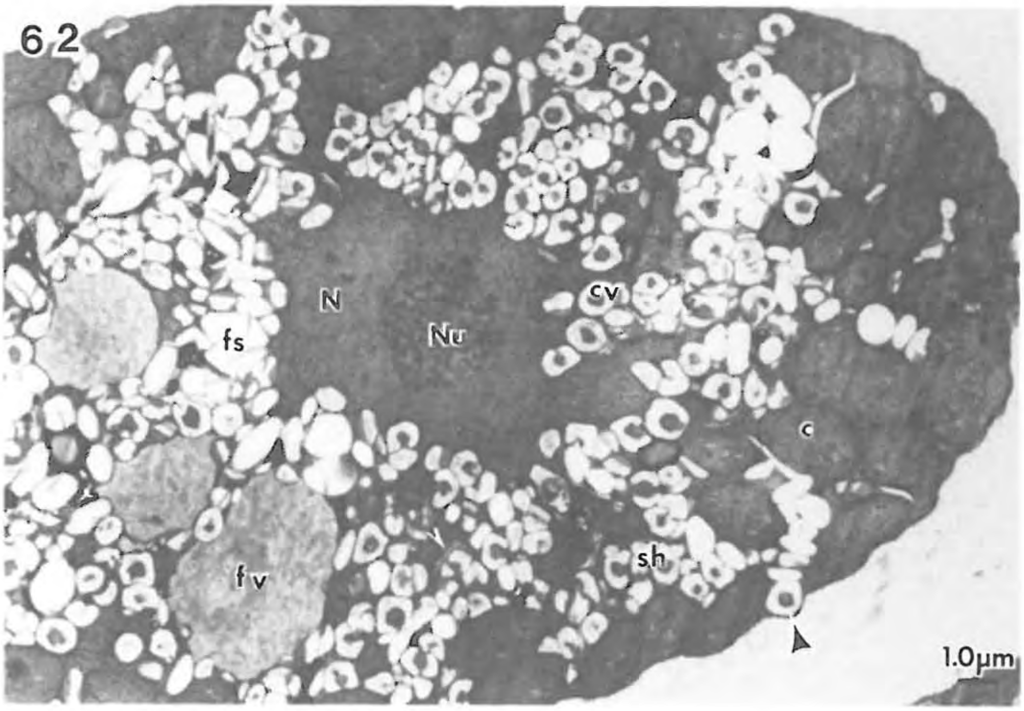

62
N
Nu
fs
cv
c
fv
sh
1.0µm

Fig. 63: Portion of a mature carpospore (TEM).

Fig. 64: Carpospore walls (TEM).

LEGEND:

cv- cored vesicle

d- dictyosome

fs- floridean starch grain

fv- fibrillar vesicle

i- inner layer

m- mitochondrion

o- outer layer

pm- plasmalemma

sh- shelled vesicle


63
fs
m
d
fv
sh
cv
1.0μm

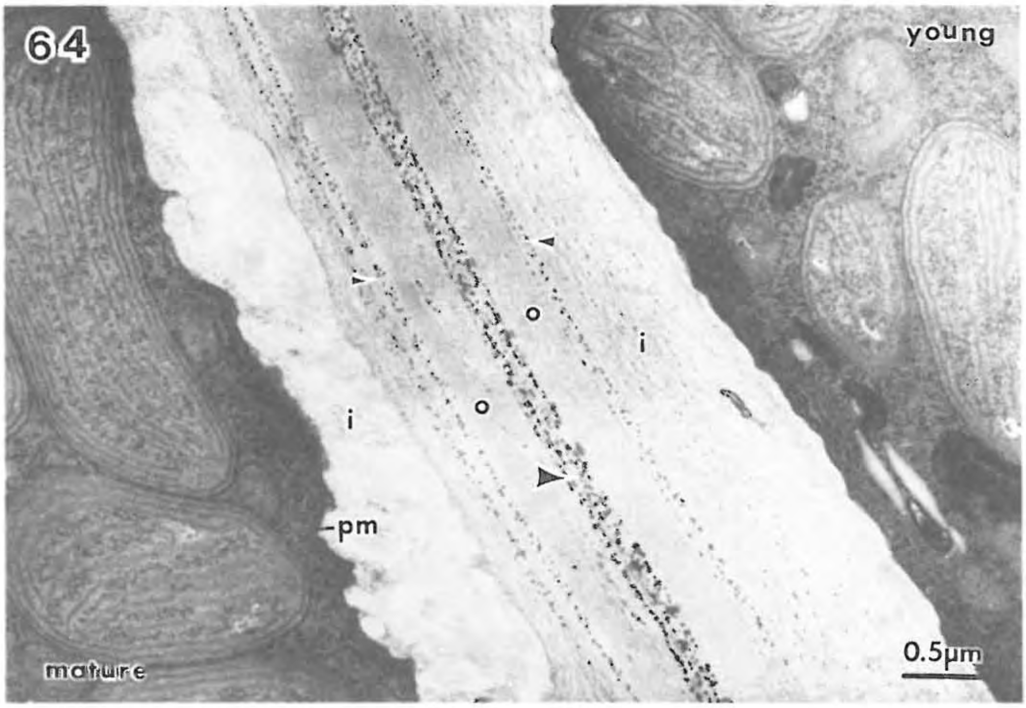

64
young
o
i
o
i
pm
mature
0.5μm

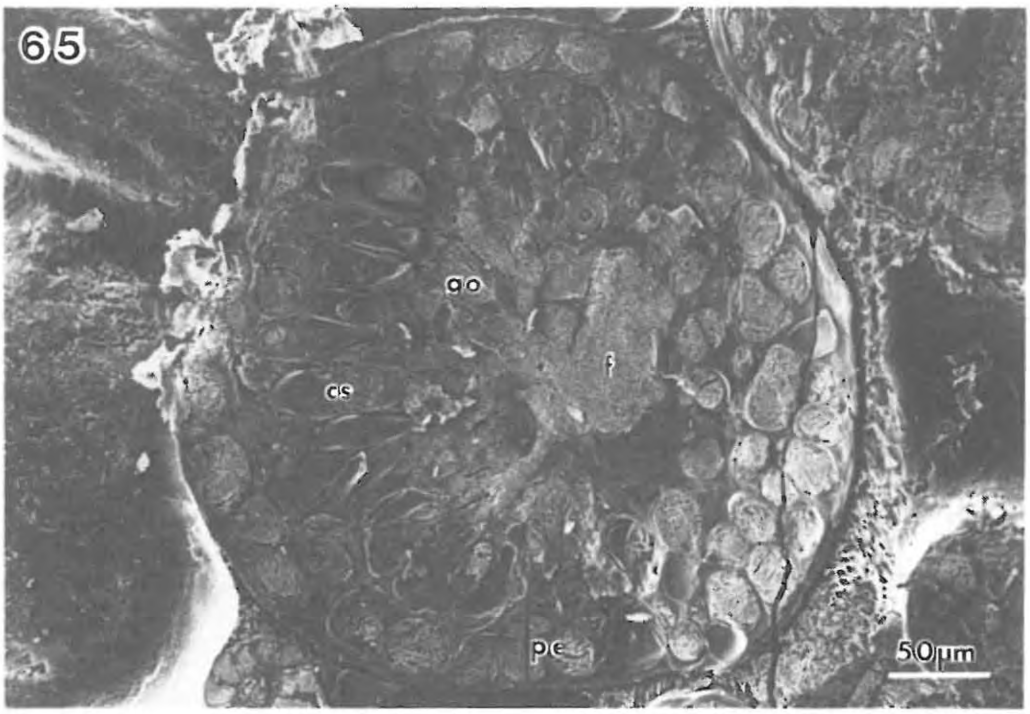
Fig. 65: Mature cystocarp (Cryo-SEM).

Fig. 66: Released carpospore (TEM).

Fig. 66 (inset): Dictyosome at the plasmalemma of a released carpospore (TEM).

LEGEND:

c- chloroplast
cs- carpospore
cv- cored vesicle
d- dictyosome
f- fusion cell
fs- floridean starch grain
fv- fibrillar vesicle
go- gonimoblast cell
m- mitochondrion
pe- pericarp
pm- plasmalemma


65
go
cs
f
pe
50µm

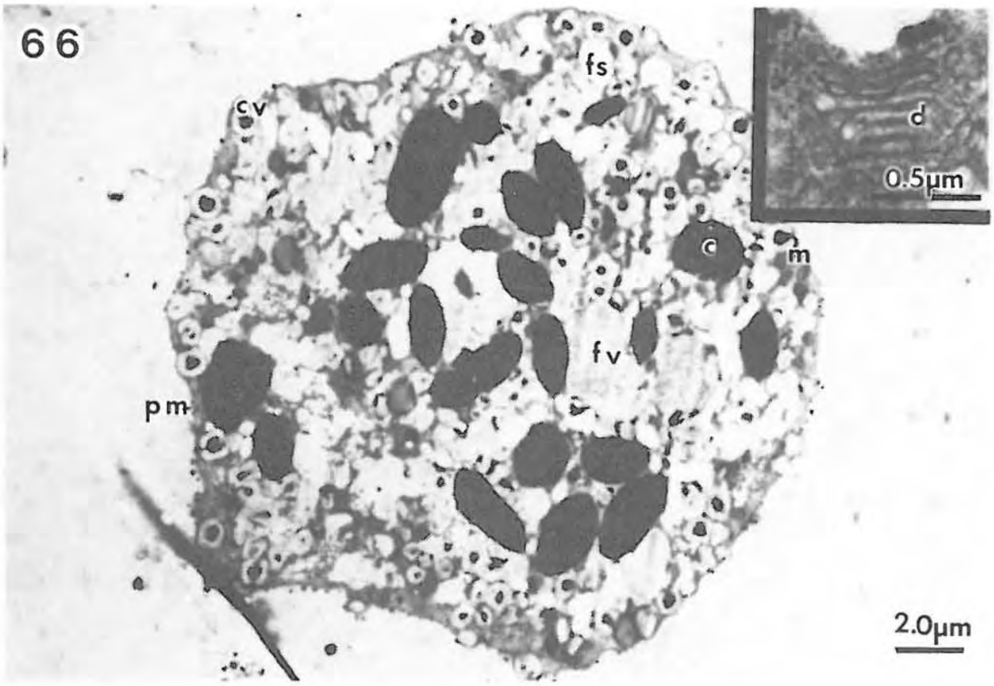
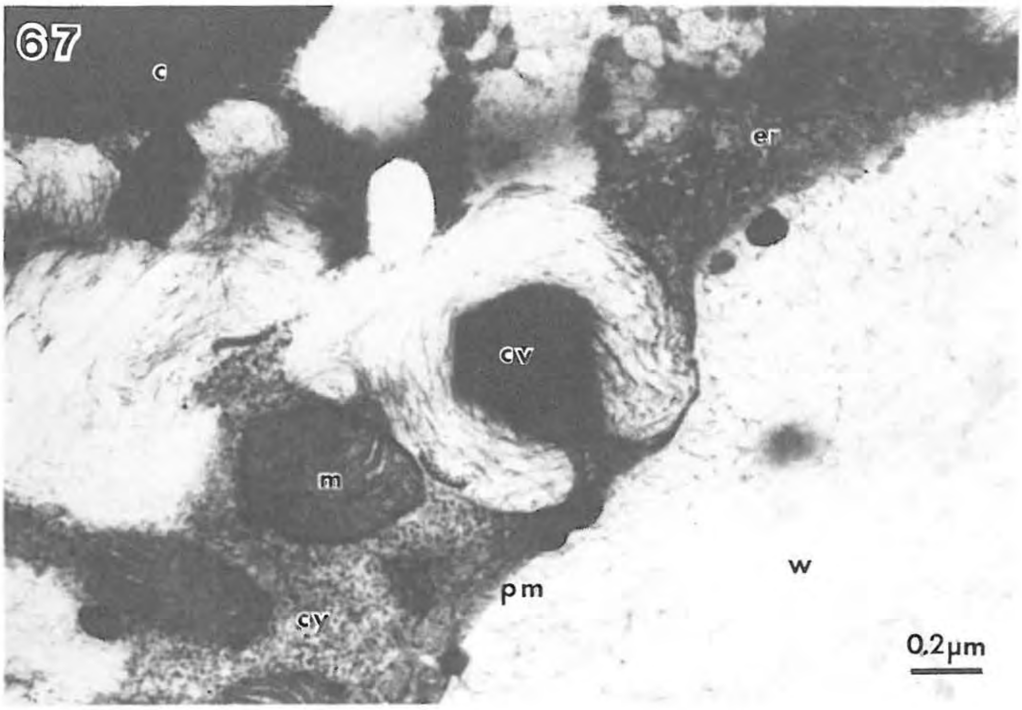

66
fs
cv
d
0.5µm
c
m
fv
pm
2.0µm

Fig. 67: Cored vesicle apparently fusing with the plasmalemma of a released carpospore (TEM).

Fig. 68: Organelles at the periphery of a released carpospore (TEM).

LEGEND:

c- chloroplast
cv- cored vesicle
cy- cytoplasm
eg- electron dense globule
er- endoplasmic reticulum
m- mitochondrion
mt- "microtubule-like" structure
pm- plasmalemma
sh- shelled vesicle
w- "swirled" inner layer


67
c
er
cv
m
pm
cy
w
0,2µm

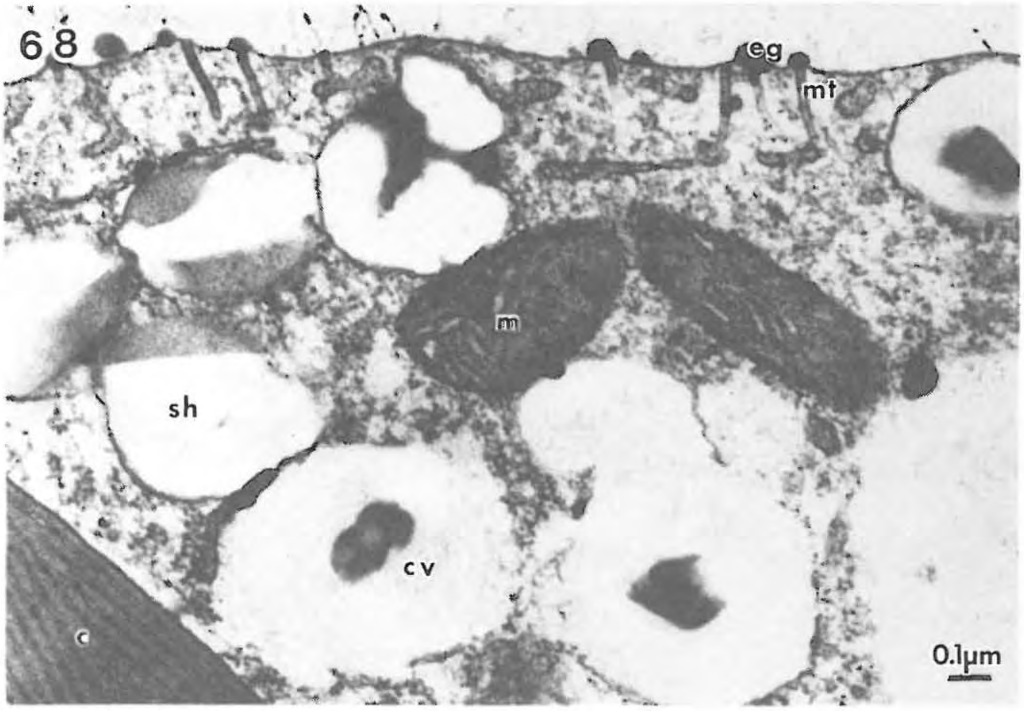

68
eg
mt
m
sh
cv
c
0.1µm

Fig. 69: Released carpospore (TEM).

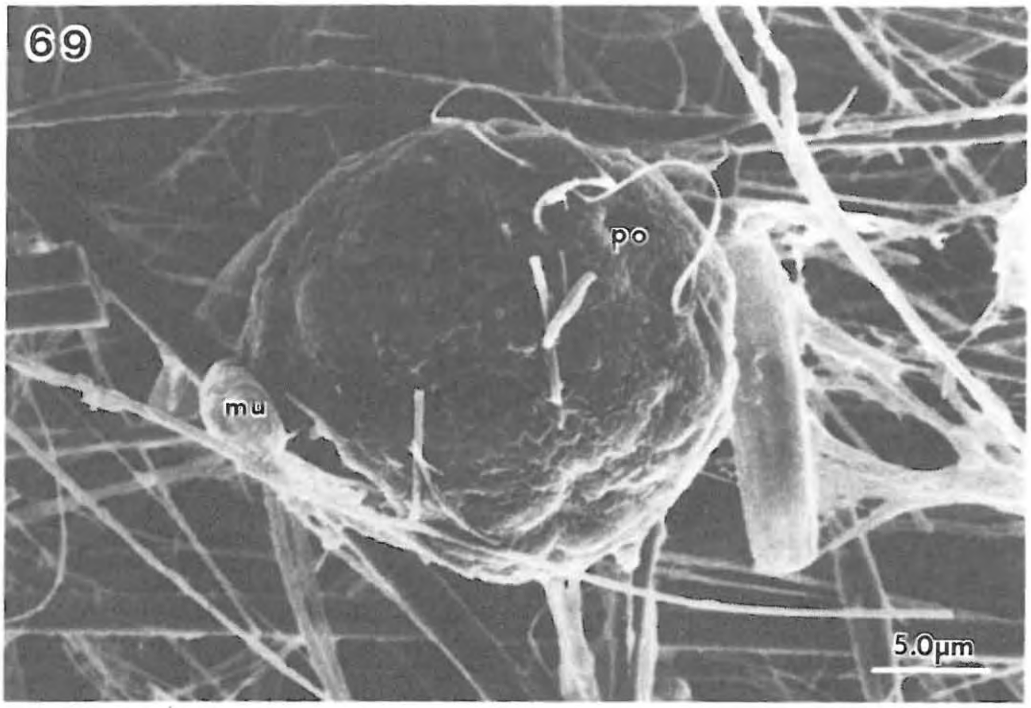
Fig. 70: Germinating carpospore (SEM).

LEGEND:

mu- mucilage

po- pore

69



70

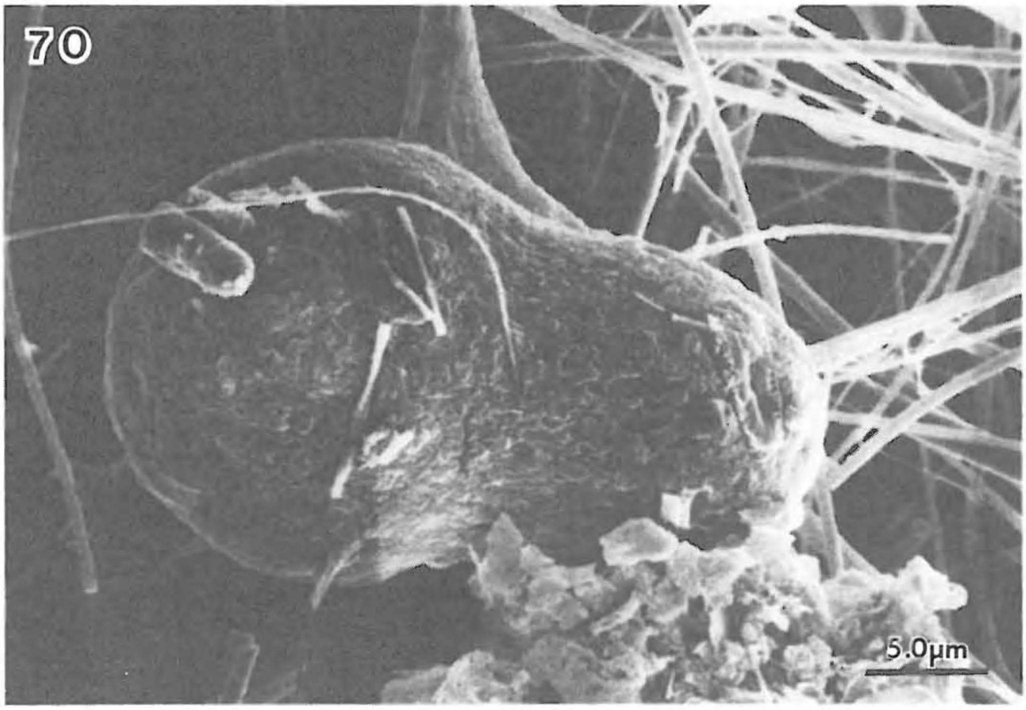


Fig. 71: Newly developed tetrasporangium (TEM).

Fig. 72: Portion of a newly developed tetrasporangium (TEM).

LEGEND:

c- chloroplast

cf-cleavage furrow

ch- chromatin

d- dictyosome

er- endoplasmic reticulum

f- fusion cell

fs- floridean starch grain

m- mitochondrion

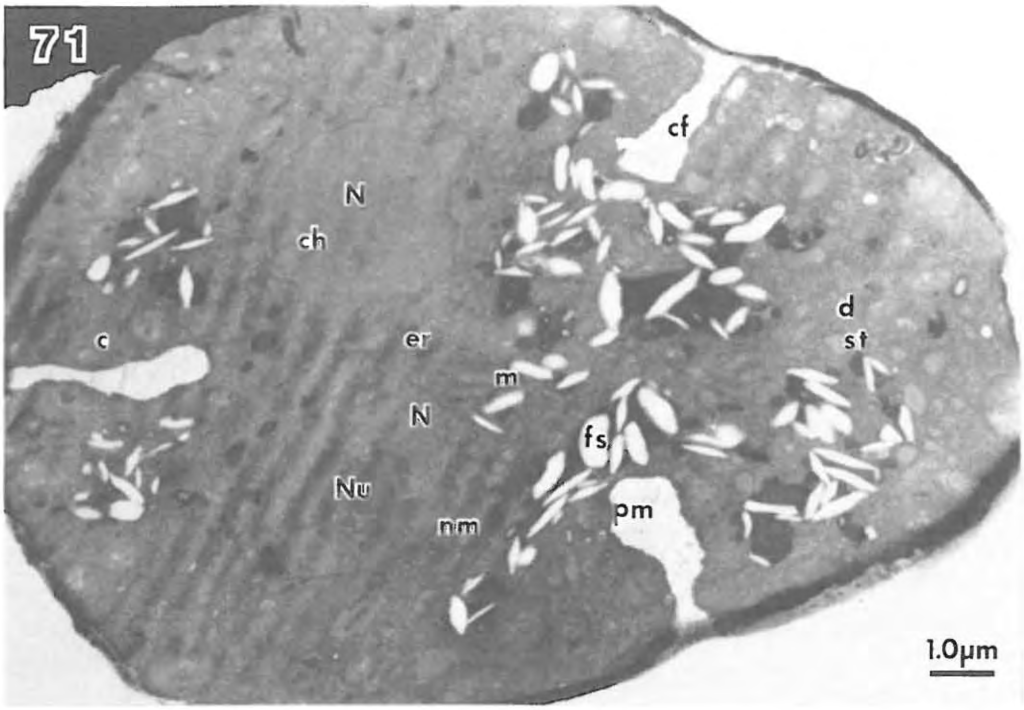
N- nucleus

nm- nuclear membrane

Nu- nucleolus

pm- plasmalemma

st- striated vesicle


71
cf
N
ch
d
st
c
er
m
N
fs
Nu
pm
nm
1.0µm

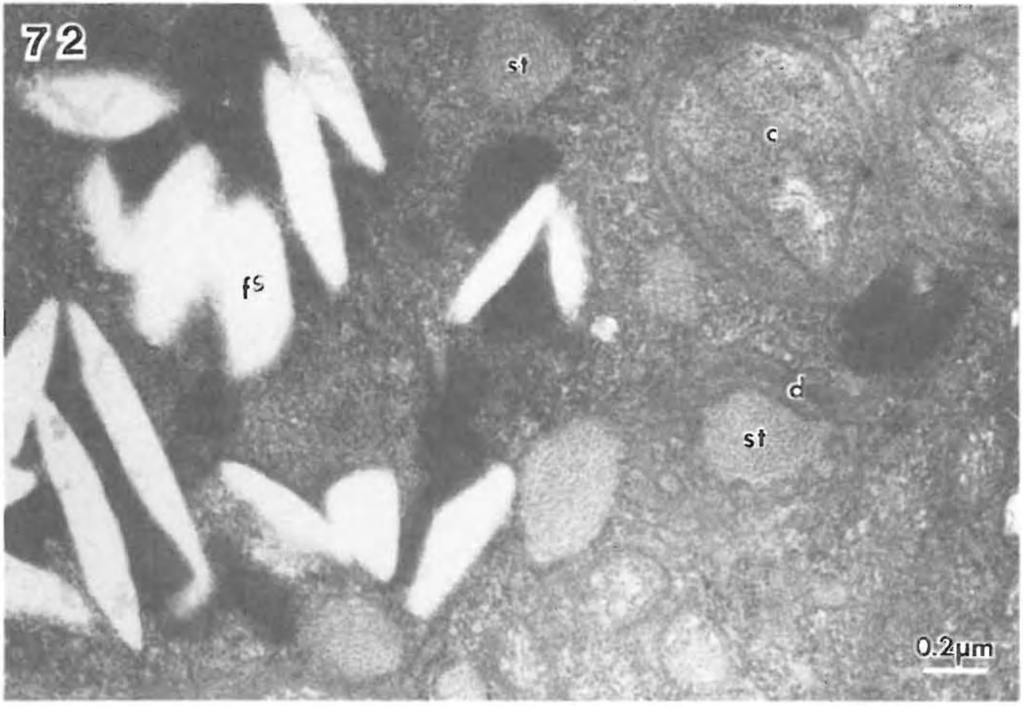

72
st
c
fs
d
st
0.2µm

Fig. 73: Portion of a newly developed tetrasporangium (TEM).

Fig. 74: Young tetrasporangium (TEM).

LEGEND:

c- chloroplast

cf- cleavage furrow

d- dictyosome

er- endoplasmic reticulum

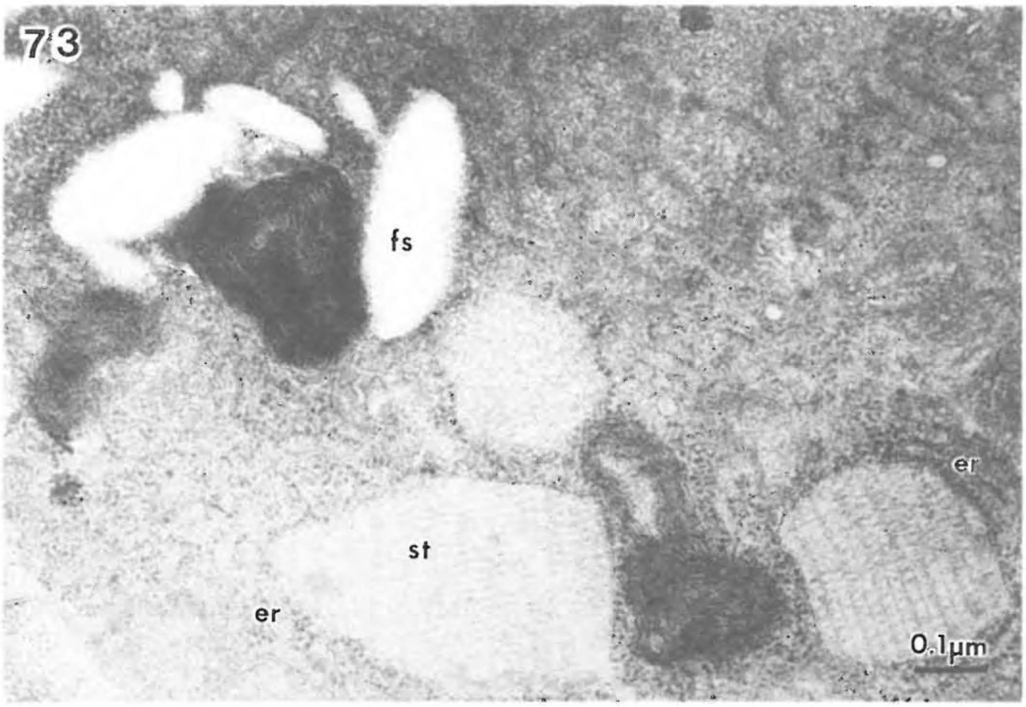
fp- fertile pericentral cell

fs- floridean starch grain

fv- fibrillar vesicle

p- pit connection

st- striated vesicle


73
fs
er
st
er
0.1μm

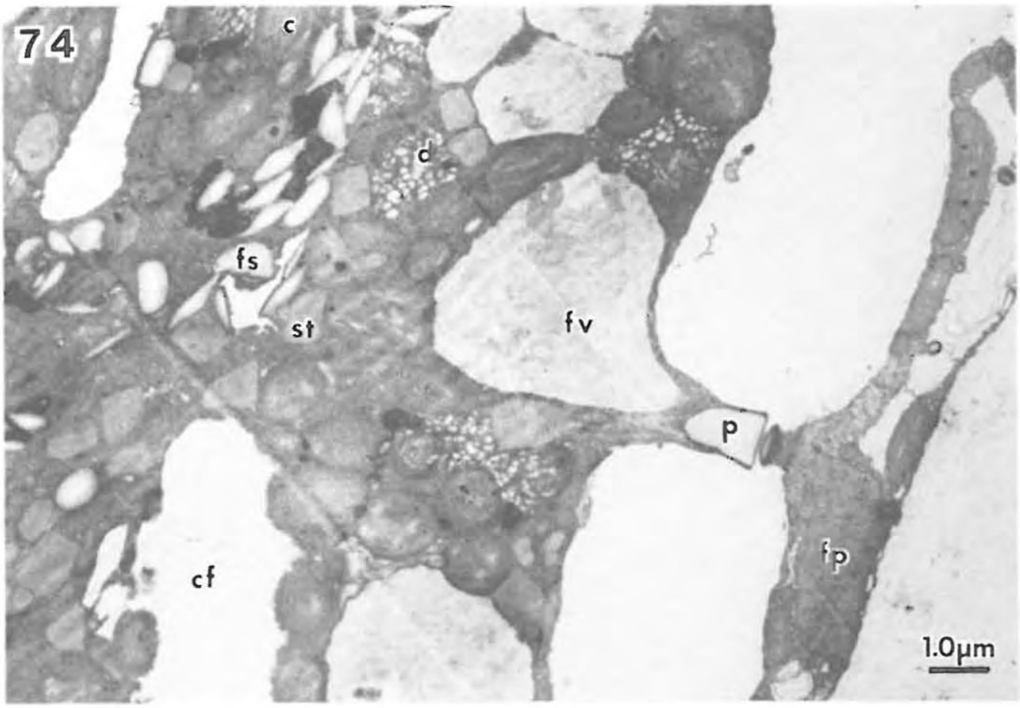
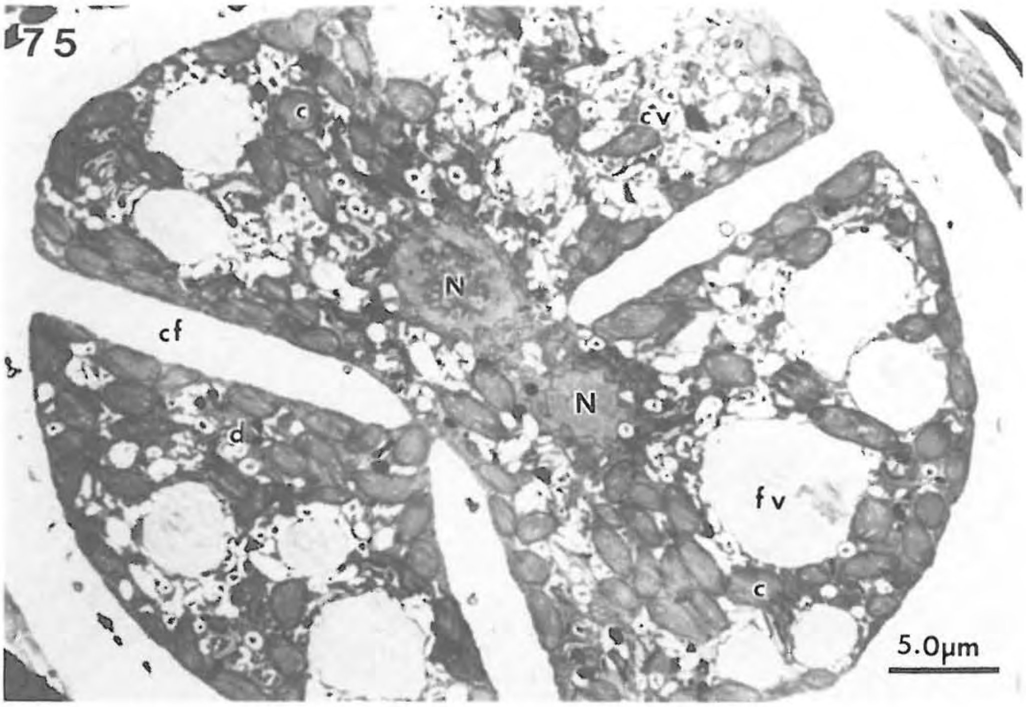

74
c
d
fs
st
fv
p
fp
cf
1.0μm

Fig. 75: Mature tetraspore (TEM).

Fig. 76: Nucleus of a released tetraspore (TEM).

LEGEND:

c- chloroplast
cf- cleavage furrow
ch- chromatin
cv- cored vesicle
d- dictyosome
fv- fibrillar vesicle
m- mitochondrion
N- nucleus
nm- nuclear membrane
Nu- nucleolus


75
c
cv
N
cf
N
d
fv
c
5.0µm

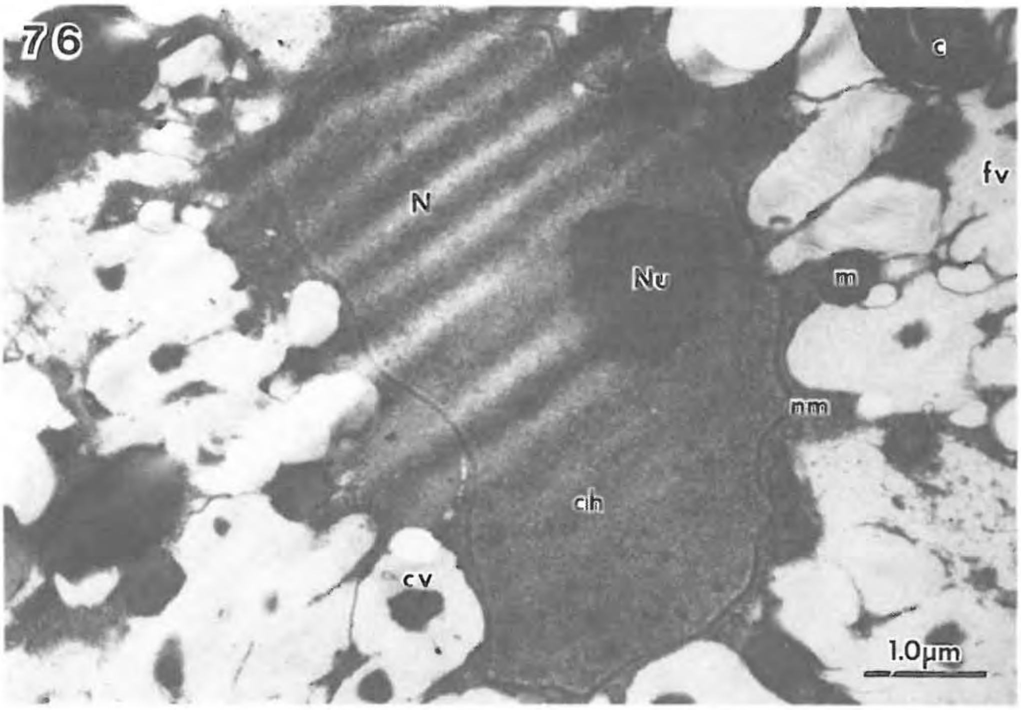
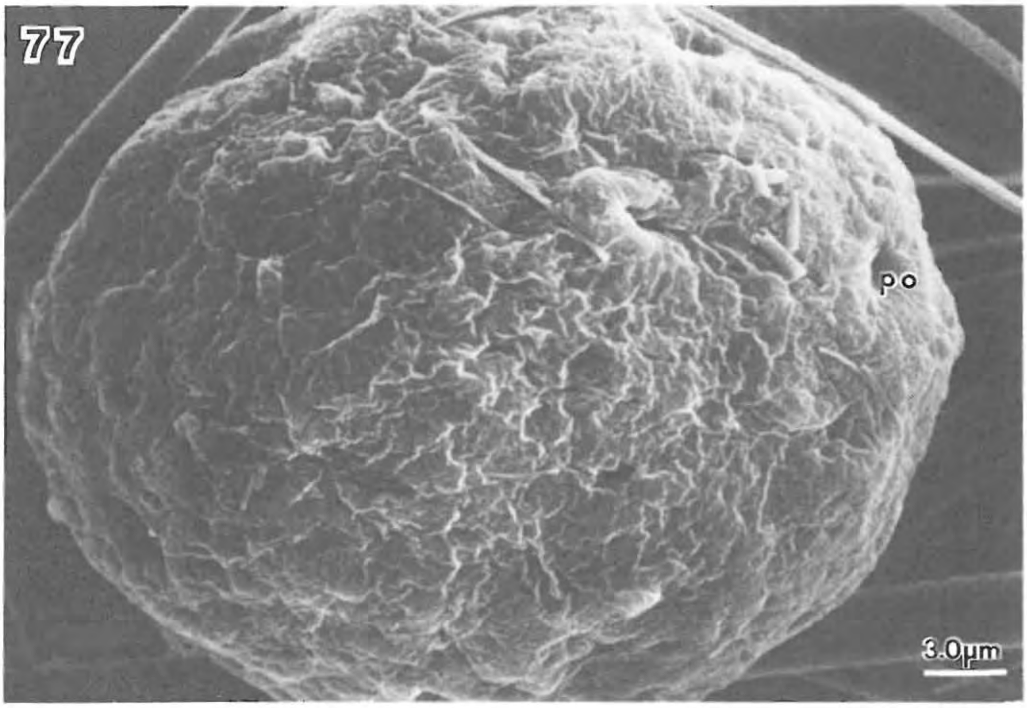

76
c
fv
N
Nu
m
nm
ch
cv
1.0µm

Fig. 77: Released tetraspore (SEM).

LEGEND:

po- pore

77



# CHAPTER 5

# CONCLUSION

The structure of Placophora binderi conforms to the basic patterns common to most Ceramiales.

The vegetative thallus contains typically Florideophycean structures: A cell wall made up of a protective cuticle, mucilage layer and a plasmalemma, and embedded in a ribosome rich cytoplasm are the chloroplasts, vacuoles, nuclei, mitochondria, endoplasmic reticulum, Golgi apparatus and Floridean starch grains.

The mature thallus exhibits primary dorsiventral symmetry in that it is established when pericentral cells and branches are initiated and not as an adaptation to the prostrate habit (Hommersand 1963).

A triphasic life history exists with isomorphic gametophyte, carposporophyte and tetrasporophyte generations. The reproductive structures develop as described by Scagel (1953) and according to typical Ceramiales patterns. The major structures peculiar to reproductive organ development are visible both at the light and the electron microscope level. In the male the spermatial vesicles, derived by endoplasmic reticulum, are seen as large clear structures in mature spermatia and their primary function is to aid in spermatial release as in Polysiphonia hendryi (Kugrens 1980).

Three vesicle types are produced in the development of carpospores and tetraspores; fibrillar vesicles, striated vesicles and cored vesicles. The latter two types are really discernible only under the electron microscope but can be seen under good resolution light microscopes (Fritsch 1935). The fibrillar vesicles are visible in later stage spores. These are dictyosome derived in *Placophora binderi* and probably act as carbohydrate storage organelles for later spore development and spore germination (Triemer and Vasconcelos 1977; Kugrens and West 1973c; Tripodi 1971). The striated vesicles appear for a short while in the earlier part of spore development. They are endoplasmic reticulum derived and may function as protein stores which are used as the spores develop. Cored vesicles are dictyosome derived in *Placophora binderi* and their major function appears to be in spore adhesion. In *Janczewskia morimotoi* cored vesicles are present in the rhizoids and the proposed function here is the rapid deposition of the spore wall to aid host penetration (Nonamura and West 1980). In *Placophora binderi* these vesicles tend to disappear as germination proceeds thus an adhesive function is likely. Scanning electron micrographs show that newly released and settled spores have tiny pores in the walls through which the cored vesicles may release their contents. The spore wall may provide initial adhesion followed by the stronger adhesive properties of the cored vesicles as in *Ceramium rubrum* (Chamberlain and Evans 1973).

The very thick mucilage layer which occurs around each spore may be derived from the cored vesicles and perhaps from the fibrillar vesicles as well. This mucilage layer may act as a protective coat against infection in germinating spores. Protection against desiccation (Boney 1981) would not be so important in Placophora binderi as spores are not moved far from the parent thalli nor does this epiphyte occur in an environment which is exposed by receding tides.

Spermatium, carpospore and tetraspore release are forceful which, is often the case in red algae and probably compensates for the lack of flagella (Boney 1981).

Spores germinate in a typical bipolar Ceramiales-type pattern (Dixon 1973).

Placophora binderi is not a parasite according to Setchell's definition as it does not form pit connections with its basiphyte nor does it have a reduced thallus or pigmentation. It is unlikely to fall within the scope of later definitions for algal parasitism which demand a derivation of materials from the host without return benefits (Evans, Callow and Callow 1978; Goff 1976).

Any nutrient transfer between Placophora binderi and its basiphytes is probably a passive process. Linskens (1963) found that Polysiphonia received some metabolites from its Codium basiphyte.

Such nutrient transfers are probably due to leakage from the old and dying Codium tissue into the space between the epiphyte and basiphyte and a subsequent diffusion directly into the thallus or via the rhizoids in both Placophora and Polysiphonia. This occurs in Zostera and Phyllospadix (Harlin 1971; 1975) which usually have epiphytes only on the older regions of their blades and a passive leakage of nutrients is proposed. When moved by water currents the basiphyte blades may also break surface films which would normally reduce nutrient diffusion into epiphytes (Harlin 1971; 1975).

Placophora binderi may be described as an "obligate epiphyte" as it was found only on algal substrates. Related species to Placophora binderi such as Amplisiphonia and Polyzonia are also all epiphytic on a range of "green", "brown" and "red" algae (Scagel 1953). Thus, within this group of algae (dorsiventral and within the subfamilies Herposiphonieae and Polyzonieae (Scagel 1953)), the epiphytic habit is a common one.

Placophora binderi is well adapted to this habit particularly on its customary basiphyte Codium. The occasional appearance on other algal basiphytes may be a result of spores landing accidentally on suitable areas for rhizoid penetration. This is probably an important point for spore survival to maturity as this species grows in an environment where wave action is high and spores may be easily washed away.

Thus Placophora binderi is probably adapted to the epiphytic habit for the reason that its customary substrate is suitable substrate in a harsh environment where competition for space is high (Harlin 1971; Moss 1982).

# APPENDIX

## WHITTMAN'S ACETO-IRON-HAEMATOXYLIN CHORAL HYDRATE STAIN (Whittman 1965)

a) Stock solution:

Mix together 4g haematoxylin, 1g iron alum ($FeNH_4(SO_4)_2 12H_2O$), 100ml 45%acetic acid. Ripen at room temperature for 2-7 days before using.

b) Working stain solution:

Add 4g chloral hydrate to 10mls of the stock solution. Dissolve overnight and centrifuge. Do not heat. Discard after one week.

c) Procedure:

Stain for two to six hours. Heat gently on a hot plate until the stain changes colour from dark brown to burnt amber. Destain with 45% acetic acid until stained as required.

## TOLUIDINE BLUE STAIN FOR THICK SECTIONS

2.5g sodium carbonate dissolved in 100mls distilled water. Dissolve 0.1g toluidine blue powder in 10mls of the sodium carbonate solution just prior to use.

## ERDSCHREIBER'S ENRICHED SEAWATER MEDIUM

1.5mM/1 $NaNO_3$; 100uM/1$NaHPO_4$; 50ml/1 sterilized soil extract to seawater.

## 5% GLUTARALDEHYDE IN FRESH SEAWATER

20mls of 25% Glutaraldehyde (ultrastructure grade) in 100mls fresh seawater.

## 0.1M pH 7.0 CACODYLATE BUFFER

Solution A: 42,8g sodium cacodylate and 1000ml distilled water.
Solution B: 10ml 36.38% concentrated hydrochloric acid and 603mls distilled water.
Mix 6.3 mls of solution B with 50 mls of solution A and then dilute to 200mls with distilled water to obtain a pH of 7.0.

## 1% CACODYLATE BUFFERED OSMIUM TETROXIDE

2% Stock Solution: Mix 2g $OsO_4$ crystals in 100mls distilled water by heating but not boiling.

1% Fixative Solution: Mix 4mls buffer solution, 10mls 2% $OsO_4$ , 2mls distilled water and 4mls 0.1N HCl.

# REFERENCES

AGHAJANIAN,J.G. and HOMMERSAND,M.H., 1978. The fine structure of the pit connections of Batrachospermum sirodotii Skuja. Protoplasma 96: 247-265.

ALLEY,C.D. and SCOTT,J.L., 1977. Unusual dictyosome morphology and vesicle formation in tetrasporangia of the marine red alga Polysiphonia denudata. J.Ultrastruct.Res. 58: 289-298.

APT,K.E., 1984. The morphology of Phaeocolax kajimurai (Rhodomelaceae, Rhodophyta), a host specific epiphyte on Lobophora variegata (Phaeophyta). Phycologia 23: 239-247.

BISALPUTRA,T. and BISALPUTRA,A-A., 1967. The occurrence of DNA fibrils in chloroplasts of Laurencia spectabilis. J.Ultrastruct.Res. 17: 14-22.

BISALPUTRA,T., RUSANOWSKI,P.C. and WALKER,W.S., 1967. Surface activity, cell wall and fine structure of pit connections in the red alga Laurencia spectabilis. J.Ultrastruct.Res. 20: 277-289.

BOLD,H.C. and WYNNE,M.J., 1978. Introduction to the algae. Structure and funtion. Prentice- Hall Inc. New Jersey.

BONEY,A.D., 1967. Carpospore release in a species of Polysiphonia. J.Nat.Hist. 4: 501-504.

BONEY,A.D., 1978. The liberation and dispersal of carpospores of the red alga Rhodymenia pertusa (Postels et Rupr.) J.Ag. J.Exp.Mar.Biol.Ecol. 32: 1-6.

BONEY,A.D., 1981. Mucilage: The ubiquitous algal attribute. British Phycol.J. 16: 115-132.

BOUCK,G.B., 1962. Chromatophore development, pits and other fine structure in the red alga, Lomentaria baileyana (Harv.) Farlow.

*J.Cell Biol.* *12*: 553-569.

BRAUNER,J.F., 1979. Developmental morphology and taxonomy of *Medeiothamnion* (Hooker f. and Harvey) comb. nov. (Ceramiaceae, Rhodophyta) from South America. *Phycologia* *18*: 338-346.

BROWN,D.L. and WEIER,T.E., 1970. Ultrastructure of the freshwater alga *Batrachospermum*. 1. Thin section and freeze-etch analysis of juvenile and photosynthetic filament vegetative cells. *Phycologia* *9*: 217-235.

CHAMBERLAIN,A.H.L. and EVANS,L.V., 1973. Aspects of spore production in the red alga *Ceramium*. *Protoplasma* *76*: 131-159.

CHEN,L.C-M., 1977. The sporophyte of *Ahnfeltia plicata* (Huds.) Fries. (Rhodophyta, Gigartinales) in culture. *Phycologia* *16*: 163-168.

CHEN.L.C-M., EDELSTEIN,T. and McLACHLAN,J., 1974. The life history of *Gigartina stellata* (Stackh.) Batt. (Rhodophyceae, Gigartinales) in culture. *Phycologia* *13*: 287-294.

CHEN,L.C-M. and McLACHLAN,J., 1972. The life history of *Chondrus crispus* in culture. *Canadian J.Bot.* *50*: 1055-1060.

CHIHARA,M., 1965. Germination of carpospores of *Bonnemaisonia nootkana*, with special reference to the life cycle. *Phycologia* *5*: 71-79.

CHIHARA,M. and KAMURA,S., 1963. On the germination of tetraspores of *Gelidiella acerosa*. *Phycologia* *3*: 69-74.

CITHAREL,J., 1972. *Polysiphonia lanosa* (L.) Tandy est-il un simple epiphyte? *Acedemie des Sciences. Comptes Rendes* *D 274*: 1904-1906.

COURT,G.J., 1980. Photosynthesis and translocation studies on Laurencia spectabilis and its symbiont Janczewskia gardneri (Rhodophyceae). J.Phycol. 16: 270-279.

DE CEW,T.C. and WEST,J.A., 1981. Life histories in the Phyllophoraceae (Rhodophyta: Gigartinales) from the Pacific coast of North America. 1. Gymnogongrus linearis and G. leptophullum . J.Phycol. 17: 240-250.

DIXON,P.S., 1973. Biology of the Rhodophyta. Oliver and Boyd. Edinburgh.

DODGE,J.D., 1973. The fine structure of algal cells. Academic Press. London.

DUCKETT,J.G. and PEEL,M.C., 1978. The role of transmission electron microscopy in elucidating the taxonomy and phylogeny of the Rhodophyta. In: IRVINE,D.E.G. and PRICE,J.H., 1978 (Eds.). Modern approaches to the taxonomy of red and brown algae. Academic Press. London.

EDELSTEIN,T., 1970. The life history of Gloiosiphonia capillaris (Hudson) Carmichael. Phycologia 9: 55-59.

EDELSTEIN,T. and McLACHLAN,J., 1971. Further observations on Gloiosiphonia capillaris (Hudson) Carmichael in culture. Phycologia 10: 215-219.

EVANS,L.V., CALLOW,J.A. and CALLOW,M.E., 1973. Structural and physiological studies on the parasitic red alga Holmsella. New Phytologist 72: 393-402.

EVANS,L.V., CALLOW,J.A. and CALLOW,M.E., 1978. Parasitic red algae: an appraisal. In: IRVINE,D.E.G. and PRICE,J.H., 1978 (Eds.) Modern approaches to the taxonomy of the red and brown algae. Academic Press. London.

FAN,K-C., 1961. Studies on Hypneocolax, with a discussion on the origin of parasitic red algae. Nova Hedwigia 3: 119-133.

FETTER,R. and NEUSHUL,M., 1981. Studies on developing and released spermatia in the red alga, Tiffaniella snyderae (Rhodophyta). J.Phycol. 17: 141-159.

FRITSCH,F.E., 1935. The structure and reproduction of the algae. Volume 2. University Press. Cambridge.

GOFF,L.J., 1976. The biology of Harveyella mirabilis (Cryptonemiales; Rhodophyceae). v. Host response to parasite infection. J.Phycol. 12: 313-328.

GOFF,L.J., 1979a. The biology of Harveyella mirabilis (Cryptonemiales; Rhodophyceae). vi. Translocation of photoassimilated $^{14}C$. J.Phycol. 15: 82-87.

GOFF,L.J., 1979b. The biology of Harveyella mirabilis (Cryptonemiales; Rhodophyceae). vii. Structure and proposed function of host-penetrating cells. J.Phycol. 15: 87-100.

GORDON-MILLS,E.M. AND WOMERSLEY,H.B.S., 1974. The morphology and life history of Mazoyerella gen.nov. (M. arachnoidea (Harvey) comb.nov.)- Rhodophyta, Ceramiaceae- from Southern Australia. Br.Phycol.J. 9: 127-137.

GUILLARD,R.R.L., 1973. Methods for microflagellates and nannoplankton. In: STEIN,J.R. (Ed.) Handbook of Phycological Methods- Culture Methods and Growth Measurments. Cambridge University Press. Cambridge.

GUIRY,M.D. and MAGGS,C.A., 1982. The morphology and life history of Dermocorynus montagnei Crouan Frat. (Halymeniaceae: Rhodophyta) from Ireland. Br.Phycol.J. 17: 215-228.

HARLIN,M.M., 1971. Epiphytic marine algae: Interactions with their hosts. Ph.D. thesis, Univ. Washington, Seattle, 184pp.

HARLIN,M.M., 1973a. "Obligate" algal epiphyte Smithora naiadum grows on synthetic substrate. J.Phycol. 9: 230-232.

HARLIN,M.M., 1973b. Transfer of products between epiphytic marine algae and host plants. J.Phycol. 9: 243-248.

HARLIN,M.M., 1975. Epiphyte-Host relationships in seagrass communities. Aquatic Botany 1: 125-131.

HARLIN,M.M. and CRAIGIE,J.S., 1975. The distribution of photosynthate in Ascophyllum nodosum as it relates to epiphytic Polysiphonia lanosa. J.Phycol. 11: 109-113.

HAWKINS,E.K., 1972. Observation on the developmental morphology and fine structure of pit connection in red algae. Cytologia 37: 759-768.

HAWKINS,E.K., 1974a. Golgi vesicles of unusual morphology and wall formation in the red alga, Polysiphonia. Protoplasma 80: 1-14.

HAWKINS,E.K., 1974b. Growth and differentiation of the Golgi apparatus in the red alga, Callithamnion roseum. J.Cell Sci. 14: 633-655.

HOMMERSAND,M.H., 1963. The morphology and classification of some Ceramiaceae and Rhodomeleceae. Univ. of Calif.Publ. in Bot. 35: 165-366.

KUGRENS,P., 1974. Light and electron microscope studies on the development and liberation of Janczewskia gardneri Setch. spermatia. (Rhodophyta). Phycologia 13: 295-306.

KUGRENS,P., 1980. Electron microscopic observations on the differentiation and release of spermatia in the marine red alga Polysiphonia hendryi (Ceramiales, Rhodomelaceae). Amer.J.Bot. 67: 519-528.

KUGRENS,P. and ARIF,I., 1981. Light and electron microscope studies on the fusion cell in *Asterocolax gardneri* Setch. (Rhodophyta, Ceramiales). *J.Phycol.* *17*: 215-223.

KUGRENS,P. and WEST,J.A., 1972a. Synaptonemal complexes in the red algae. *J.Phycol.* *8*: 187-191.

KUGRENS,P. and WEST,J.A., 1972b. Ultrastructure of spermatial development in the parasitic red algae *Levringiella gardneri* and *Erythrocystis saccata*. *J.Phycol.* *8*: 331.343.

KUGRENS,P. and WEST,J.A., 1972c. Ultrastructure of tetrasporogenesis in the parasitic red alga *Levringiella gardneri* (Setchell) Kylin. *J.Phycol.* *8*: 370-383.

KUGRENS,P. and WEST,J.A., 1973a. The ultrastructure of carpospore differentiation in the parasitic red alga *Levringiella gardneri* ( Setch.) Kylin. *Phycologia* *12*: 163-173.

KUGRENS,P. and WEST,J.A., 1973b. The ultrastructure of an alloparasitic red alga *Choreocolax polysiphoniae*. *Phycologia* *12*: 175-186.

KUGRENS,P. and WEST,J.A., 1974. The ultrastructure of carposporogenesis in the marine hemiparasitic red alga *Erythrocystis saccata*. *J.Phycol.* *10*: 139-147.

LEE,R.E., 1971. The pit connections of some lower red algae; Ultrastructure and phylogenetic significance. *Br.Phycol.J.* *6*: 29-38.

LEEDALE,G.F., 1970. Phylogenetic aspects of nuclear cytology in the algae. *Ann.N.Y.Acad.Sci.* *175*: 429-453.

LINSKENS,H., 1963. Beitrag zur frage der bezeihagen zwischen epiphyt und basiphyt bei marinen algen. *Pubbl.Staz.Zool.Napoli* *33*: 274-293.

MAGRUDER,W.H., 1977. The life history of the red algal genus Ahnfeltia concinna (Rhodophyta, Gigartinales). Phycologia 16: 197-203.

MARKHAM,J.W. and HAGMEIER,E., 1982. Observations and effects of germanium dioxide on the growth of macro-algae and diatoms. Phycologia 21: 125-130.

MCDONALD,K., 1972. The ultrastructure of mitosis in the marine red alga Membranoptera platyphylla. J.Phycol. 8: 156-166.

McLACHLAN,J., 1973. Growth media- marine. In: STEIN,J.R. (Ed.) Handbook of phycological methods and growth requirements. Cambridge Univ. Press. Cambridge.

McROY,C.P. and GOERING,J.J., 1974. Nutrient transfer between the seagrass Zostera marina and its epiphytes. Nature 248: 173-174.

MOLLENHAUER,H.H. and MORRÉ,D.J., 1966. Golgi apparatus and plant secretion. Ann.Rev.Plant Physiol. 17: 27-46.

MOSS,B.L., 1982. The control of epiphytes by Halidrys siliquosa (L.) Lyngb. (Phaeophyta, Cystoseiraceae). Phycologia 21: 185-191.

NGAN,Y. and PRICE,I.R., 1983. Periodicity of spore discharge in tropical Florideophyceae (Rhodophyta). Br.Phycol.J. 18: 83-95.

NONOMURA,A.M., 1979. Development of Janczewskia morimotoi (Ceramiales) on its host Laurencia nipponica (Ceramiales, Rhodophyceae). J.Phycol. 15: 154-162.

NONAMURA,A.M. and WEST,J.A., 1980. Ultrastructure of the parasite Janczewskia morimotoi and its host Laurencia nipponica (Ceramiales, Rhodophyta). J.Ultrastruct.Res. 73: 183-198.

OGATA,E., MATSUI,T. and NAKAMURA,H., 1972. The life cycle of Gracilaria verrucosa (Rhodophyceae, gigartinales) in vitro. Phycologia 11: 75-80.

PEEL,M.C. and DUCKETT,J.G., 1975. Studies of spermatogenesis in the Rhodophyta. In: DUCKETT,J.G. and RACEY,P.A. (Eds.). The biology of the male gamete. Academic Press. London.

PEYRIERE,M., 1970. Evolution de l'appareil de Golgi au cours de la tetrasporogenese de Griffithisia flosculosa (Rhodophycee). C.R.Acad.Sc.Paris. D 270: 2071-2074.

PEYRIERE,M., 1971. Etude infrastructurale des spermatocystes du Griffithisis flosculosa (Rhodophycee). C.R.Acad.Sc.Paris. D273: 2071-1074

PEYRIÈRE,M., 1974. Etude infrastructurale des spermatocystes et spermaties de differentes Rhodophycees floridees. C.R.Acad.Sc.Paris D278: 1019-1022.

POLANSHEK,A.R. and WEST,J.A., 1975. Culture and hybridisation studies on Petrocelis (Rhodophyta) from Alaska and California. J.Phycol. 11: 434-439.

POLANSHEK,A.R. and WEST,J.A., 1977. Culture and hybridisation studies on Gigartina papillata (Rhodophyta). J.Phycol. 13: 141-149.

PRINCE,J.S., 1979. The life cycle of Crouania pleonospora Taylor (Rhodophyta, ceramiales) in culture. Phycologia 18: 247-250.

PROVASOLI,L., McLAUGHLIN,J.J.A. and DROOP,M.R., 1957. The development of artificial media for marine algae. Archiv.für. Mikrobiologie BD.25. 5: 392-428.

PRUD'HOMME,VAN REINE,W.F., SLUIMAN,H,J. and MARCHAND,R.P., 1983. Red algae on European salt marshes II. Catanella caespitosa (Rhabdoniaceae). Aquatic Bot. 15: 287-298.

PUESCHEL,C.M., 1977. A freeze-etch study of the ultrastructure of red algal pit plugs. Protoplasma 91: 15-30.

PUESCHEL,C.M., 1979. Ultrastructure of tetrasporogenesis in Palmaria palmata (Rhodophyta). J.Phycol. 15: 409-424.

PUESCHEL,C.M., 1980a. A reappraisal of the cytochemical properties of Rhodophycean pit plugs. Phycologia 19: 210-217.

PUESCHEL,C.M., 1980b. Pit connections and translocation in red algae. Science 209: 422-423.

PUESCHEL,C.M., 1982. Ultrastructural observations of tetrasporangia and conceptacles in Hildenbrandia (Rhodophyta: Hildenbrandiales). Br.Phycol.J. 17: 333-341.

PUESCHEL,C.M. and COLE,K.M., 1982. Rhodophycean pit plugs: an ultrastructural survey with taxonomic implications. Amer.J.Bot. 69: 703-720.

QUIRK,H.M. and WETHERBEE,R., 1980. Structural studies on the host-parasite relationship between the red algae Holmsella and Gracilaria. Micron 11: 511-512.

RAMM-ANDERSON,S.M. and WETHERBEE,R., 1982. Structure and development of the carposporophyte of Nemalion helminthoides (Nemalionales, Rhodophyta). J.Phycol. 18: 133-141.

RAMUS,J., 1969a. Dimorphic pit connections in the red alga Pseudogloiophloea. J.Cell Biol. 41: 340-345.

RAMUS,J., 1969b. Pit connection formation in the red alga Pseudogloiophloea. J.Phycol. 5: 57-63.

RAMUS,J., 1971. Properties of septal plugs from the red alga Griffithisia pacifica. Phycologia 10: 99-103.

RAMUS,J. and ROBINS,D.M., 1975. The correlation of Golgi activity and polysaccharide secretion in Porphyridium. J.Phycol. 11: 70-74.

REYNOLDS,E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J.Cell Biol. 17: 208-212.

SCAGEL,R.F., 1953. A morphological study of some dorsiventral Rhodomelaceae. Univ.Calif.Publ. in Bot. 27: 1-108.

SCOTT,J., BOSCO,C., SCHORNSTEIN,K. and THOMAS,J., 1980. Ultrastructure of cell division and reproductive differentiation of male plants in the Florideophycidae (Rhodophyta): Cell division in Polysiphonia. J.Phycol. 16: 507-524.

SCOTT,J.L. and DIXON,P.S., 1973a. Ultrastructure of tetrasporogenesis in the marine red alga Ptilota hypnoides. J.Phycol. 9: 29-46.

SCOTT,J.L. and DIXON,P.S., 1973b. Ultrastructure of spermatium liberation in the marine red alga Ptilota densa. J.Phycol. 9: 85-91.

SEAGRIEF,S.C., 1967. The seaweeds of the Tsitsikama coastal national park. National Parks Board of the Republic of South Africa.

SEAGRIEF,S.C., 1980. Seaweeds of Maputoland. In: BRUTON,M.N. and COOPER,K.H. (Eds.). Studies on the ecology of Maputoland. Rhodes University and the Natal branch of the Wildlife Society of Southern Africa.

SEAGRIEF,S.C., 1984. A catalogue of South African green, brown and red marine algae. Memoirs of the Bot.Survey of S.Afr. 47: 1-72.

SETCHELL,W.A., 1918. Parasitism among red algae. Am.Phil.Soc.Proc. 57: 155-172.

SHEATH,R.G., HELLEBUST,J.A. AND SAWA,T., 1981. Ultrastructure of the Floridean starch granule. Phycologia 20: 292-297.

SHEVLIN,D.E. and POLANSHEK,A.R., 1978. Life history of Bonnemaisonia geniculata (Rhodophyta): A laboratory and field study.

J.Phycol. 14: 282-298.

SOUTH,G.R., 1968. Carpospore germination and the life history of Cumagloia andersonni: Some observations from culture. Can.J.Bot. 46: 1463-1466.

STEGENGA,H., 1978. The life history of Rhodochorton purpureum and Rhodochorton floridulum (Rhodophyta, Nemalionales) in culture. Br.Phycol.J. 13: 279;289.

SUNESON,S., 1982. The culture of bisporangial plants of Dermatilithon litorale (Suneson) Hamel et Lemoine (Rhodophyta, Corallinaceae). Br.Phycol.J. 17: 107-116.

SWALE,E.M.F. and BELCHER,J.H., 1963. Morphological observations on wild and cultured material of Rhodochorton investiens (Lenormand) nov.comb. (Balbiana investians (Lenorm.) Sirodot). Ann.Bot. 27: 281-290.

TRIEMER,R.E. and VASCONCELOS,A.C., 1977. The ultrastructure of carposporogenesis in Caloglossa leprierii (Delesseriaceae, Ceramiales). Am.J.Bot. 64: 825-834.

TRIPODI,G., 1971. Some observations on the ultrastructure of the red alga Pterocladia capillacea (Gmel.) Born et Thur. J.Submicros. Cytol. 3: 63-70.

TURNER,C.H.C. and EVANS,L.V., 1978. Translocation of photoassimilated $^{14}C$ in the red alga Polysiphonia lanosa. Br.Phycol.J. 13: 51-55.

TVETER,E. and MATHIESON,A.C., 1976. Sporeling coalescence in Chondrus crispus (Rhodophyceae). J.Phycol. 12: 110-118.

TVETER-GALAGHER,E., MATHIESON,A.C. and CHENEY,D.P., 1980. Ecology and development of male plants of Chondrus crispus (Gigartinales, Rhodophyta). J.Phycol.16: 257-264.

WAALAND,J.R. and KEMP, C.I., 1972. Observations on the life history of *Membranoptera multiramosa* Gardner (Rhodophyceae, Ceramiales) in culture. *Phycologia* *11*: 15-18.

WEST,J.A., 1970. The life history of *Rhodochorton concrescens* in culture. *Br.Phycol.J.* *5*: 179;186.

WEST,J.A., 1972. The life history of *Petrocelis franciscana*. *Br.Phycol.J.* *7*: 299-308.

WEST,J.A., POLANSHEK,A.R. and SHEVLIN,D.E., 1978. Field and culture studies on *Gigartina agardhii* (Rhodophyta). *J.Phycol.* *14*: 416-426.

WETHERBEE,R., 1978. Differentiation and continuity of the Golgi apparatus during carposporogenesis in *Polysiphonia* (Rhodophyta). *Protoplasma* *95*: 347-360.

WETHERBEE,R., 1979. "Transfer connections": Specialised pathways for nutrient translocation in a red alga? *Science* *204*: 858-859.

WETHERBEE,R., 1980. Postfertilization development in the red alga *Polysiphonia* 1. Proliferation of the carposporophyte. *J.Ultrastruct.Res.* *70*: 259-274.

WETHERBEE,R.and SCOTT,F.J., 1980. The fine structure and distribution of "transfer connections" in the red alga *Polysiphonia.* *Micron* *11*: 509-510.

WETHERBEE,R. and WEST.J.A., 1976. Unique Golgi apparatus and vesicle formation in a red alga. *Nature* *259*: 566-567.

WETHERBEE,R. and WEST,J.A., 1977. Golgi apparatus of unique morphology during early carposporogenesis in a red alga. *J.Ultrastruct.Res.* *58*: 119-133.

WETHERBEE,R. and WYNNE,M.J., 1973. The fine structure of the nucleus and nuclear associations of developing carposporangia in *Polysiphonia novae-angliae* (Rhodophyta). *J.Phycol.* *9*: 402-407.

WHITTICK,A., 1981. Culture and field studies on *Callithamnion hookeri* (Dillw.) S.F.Grey (Rhodophyta; Ceramiaceae) from Newfoundland. *Br.Phycol.J.* *16*: 289-295.

WHITTICK,A. and WEST,J.A., 1979. The life history of a monoecious species of *Callithamnion* (Rhodophyta, Ceramiaceae) in culture. *Phycologia* *18*: 30-37.

WITTMANN,W., 1965. Aceto-Iron-Haematoxylin-Chloral Hydrate for chromasome staining. *Stain Tech.* *40*: 161-164.

WOLLASTON,E.M., 1974. Sexual reproduction in *Balli mariana* Harvey and *Ballia ballioides* (Sonder) Wollaston (Ceramiaceae. Rhodophyta). *Phycologia* *13*: 21-26.

WOOD,D.D. and LUCK,D.J.L., 1971. A paracrystalline inclusion in *Neurospora crassa*. *J.Cell Biol.* *51*: 249-264.

YOUNG,D.N. AND WEST,J.A., 1979. Fine structure and histochemistry of vesicle cells of the red alga *Antithamnion defectum* (ceramiaceae). *J.Phycol.* *15*: 49-57.