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STUDIES ON SOME BRITISH COLUMBIAN REPRESENTATIVES OF THE
ERYTHROPELTIDACEAE (RHODOPHYCEAE, BANGIOPHYCIDAE)

by

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ABSTRACT

Four species of the Erythropeltidaceae [Smithora naiadum (Anderson) Hollenberg, Erythrotrichia carnea (Dillwyn) J. Agardh, Erythrotrichia boryana (Montagne) Berthold and Erythrotrichia pulvinata Gardner] were observed in freshly collected and cultured conditions using light and electron microscopic techniques. The North American Pacific coast distribution of these algae was revised in view of recent collections in British Columbia and Alaska by various workers. A study of their morphologies and life histories revealed new information concerning production of asexual reproductive units (monospores) from the basal attachment organs of E. pulvinata and S. naiadum.

At an ultrastructural level, many organelles in the vegetative cells of the Erythropeltidaceae examined were found to be similar to those reported in other members of the Rhodophyceae. However, several interesting fine structural characteristics were noted. The cellular shape was remarkably irregular, exhibiting many cytoplasmic protrusions into the cell wall. The single lobed chloroplast possessed a uniform lamellar arrangement and primitive thylakoid stacks or bands. In addition, multivesicular bodies occurred within the cytoplasm and in the cell wall near the plasmalemma. There was no evidence of any type of intercellular connection. The vegetative cell ultrastructure of E. boryana and E. pulvinata was virtually identical to S. naiadum but E. carnea exhibited fewer pyrenoid-traversing lamellae and a somewhat different cell wall morphology.

Monospore differentiation and release in the Erythropeltidaceae was found to involve a number of specialized subcellular activities. Concomitant with a rounding of the protoplast and reduction in vacuolar area in the vegetative cell, was the accumulation of two products originating from dictyosomes. The possible functions of these products are discussed in relation to spore

release and attachment. Additional fine structural features of the developing monospore included an increased number of mitochondria and nuclear pores, a large amount of endoplasmic reticulum and an association between the chloroplast and the nuclear envelope. Upon release the monospore lacked a cell wall and was typified by an extensive accumulation of dictyosome product. In addition, the chloroplast exhibited a "pseudogranum-like" arrangement of thylakoids. The ultrastructural aspects of monospore degeneration in culture are also described.

Monospore germination in S. naiadum involved several cellular changes including formation of a cell wall and a number of vacuoles. A large amount of peripheral endoplasmic reticulum and certain dictyosome populations appeared to play an important role in wall construction while other populations of dictyosomes appeared to be involved in vacuole formation. Of special interest, since it has not been reported in the Rhodophyceae, was the occurrence of a crystalline matrix in some pyrenoids. In addition, the presence of microtubular spindle fibres was demonstrated. The alternate methods of holdfast formation in this alga are also discussed.

Sexual reproduction in the Erythropeltidaceae is poorly known. In this study, an ultrastructural description of "spermatial" production in S. naiadum is presented. The dictyosome appeared to play an important role in the maturation of these pale cells. Evidence of the process of gametogenesis and fertilization in E. boryana is also shown.

In a concluding discussion, certain ultrastructural and gross morphological information is employed in the construction of a bilateral scheme on the evolution of growth types in the Bangiophycidae.

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I. PREFACE

The Rhodophyceae are taxonomically separated into two subclasses: the Florideophycidae and the less advanced Bangiophycidae. This latter group is subdivided into several orders, one of which, the Bangiales, contains two families: the Bangiaceae and the Erythropeltidaceae. The Erythropeltidaceae Skuja are distinguished by the following characteristics: marine; filamentous or nonfilamentous thalli attached by a rhizoidal or cushion-like holdfast; cells containing a single stellate or parietal chloroplast with a centrally located pyrenoid; monospores and/or distinctive, pale, crescent-shaped cells may be formed from asymmetrical divisions of vegetative cells.

This family has achieved world wide distribution and in certain restricted habitats a particular species may become the dominant algal form. There are four genera represented on the Pacific coast of North America: Erythrocladia, Erythrotrichia, Porphyropsis and Smithora. The species chosen for this study were Erythrotrichia carnea (Dillwyn) J. Agardh, Erythrotrichia boryana (Montagne) Berthold, Erythrotrichia pulvinata Gardner and Smithora naiadum (Anderson) Hollenberg.

Collectively, the chosen plants illustrate a maximum morphological variability within the group and provide an interesting sequence of increasing structural complexity. E. carnea, the simplest, possesses a uniseriate thallus with a rhizoidal attachment, E. boryana features multiseriate flat thalli arising from a monostromatic disc-like holdfast, E. pulvinata is typified by multiseriate flat thalli originating from a multistromatic basal holdfast and Smithora features larger, monostromatic to distromatic blades attached by a multistromatic basal cushion (see Pl. I).

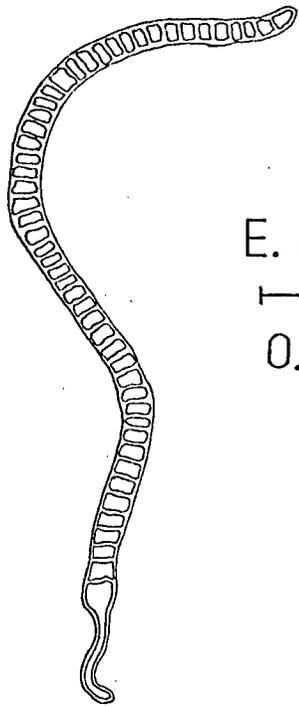
The taxonomy of the Erythropeltidaceae is in a somewhat confused state.

Heerøbut (1968) has attempted to correct this situation but his efforts have been severely criticized by certain authors (e.g. Dangeard, 1969). In addition, as Drew (1956) has pointed out: "... our knowledge of the reproductive processes and the life history of many of these algae is very scanty; consequently the need for precise and well documented investigations is stressed." This situation has undoubtedly been precipitated by such factors as the scarcity of many species, the epiphytic nature of others and the relatively small size of the majority of the representatives. Refined laboratory culture techniques and the use of the electron microscope have proved helpful in surmounting such barriers in recent years. These tools were applied in the present study in an attempt to add to existing information on this family.

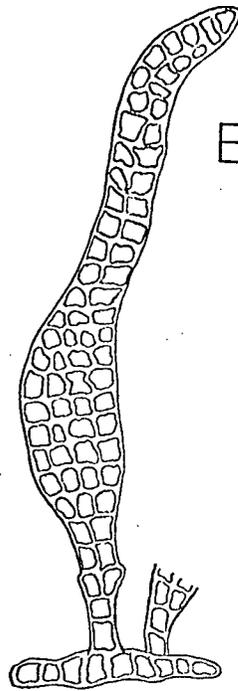
Plate I.

Scale drawings of the species of Erythropeltidaceae used in this study:

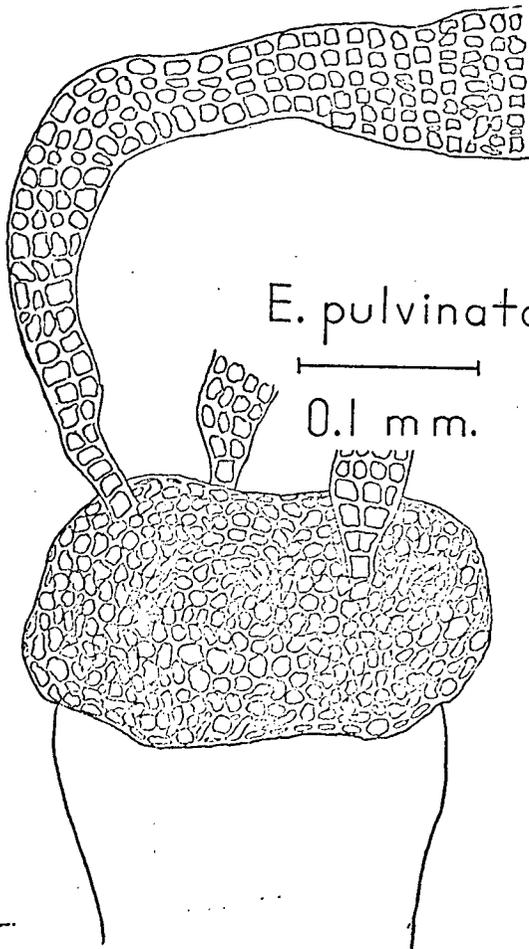
Erythrotrichia carnea, Erythrotrichia boryana, Erythrotrichia pulvinata
and Smithora naiadum.



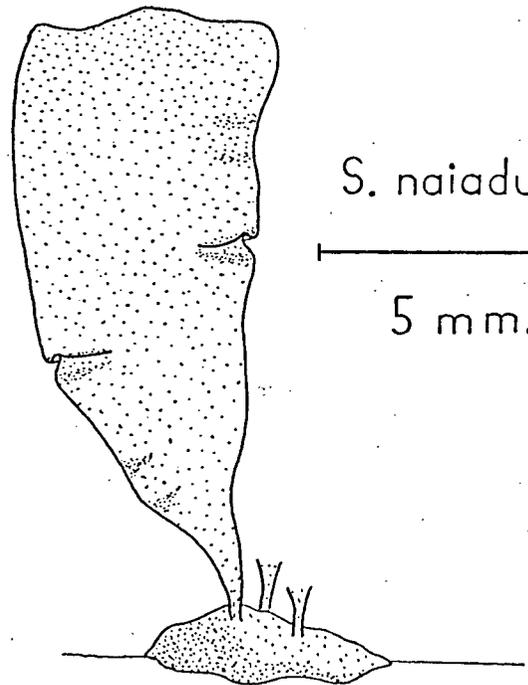
E. carnea
0.1 mm.



E. boryana
0.1 mm.



E. pulvinata
0.1 mm.



S. naiadum
5 mm.

II. MATERIALS AND METHODS

Smithora naiadum was collected intertidally at Stanley Park, Vancouver, British Columbia (Lat. 49°19' N., Long. 123°09' W.), Sooke, Vancouver Island, British Columbia (Lat. 48°21' N., Long. 123°43' W.) and Point No Point (Glacier Point), Vancouver Island, British Columbia (Lat. 48°23' N., Long. 123°59' W.). All species of Erythrotrichia were collected at the latter site. At these locations material is most plentiful during the months of May to October in the lower and middle intertidal zones. Smithora is a specific epiphyte on the sea grasses Phyllospadix scouleri and Zostera marina. In the collection area E. boxyana is also epiphytic on these marine seed plants and on Smithora. Both E. carnea and E. pulvinata were found attached to the green alga Codium fragile. Monosporic plants were available during periods of low tides from June to October while "spermatangial" Smithora was collected from August to October.

Freshly collected plants were stored on ice while being transported to the laboratory, then were isolated from host material, washed carefully and placed in plastic Petri dishes containing a modified Erdschreiber medium (sea water- 1.0 l., NaNO₃- 200 mg., Na₂HPO₄·7H₂O- 20 mg., KNO₃- 50 mg., Fe(EDTA)- 1 mg., TRIS- 500 mg., soil H₂O- 50 ml., vitamin B12- 2 mg., GeO₂- 10 mg.). Cultures were kept in a constant temperature incubator at 12°C under a 12 hr./12 hr. light regime at 700-800 lux (fluorescent light). When culturing germinating monospores, agitation of the culture dishes was kept at a minimum resulting in many of these structures adhering to parts of the parent blade. The use of the thalli as a substrate in this manner facilitated preparation of the material for electron microscopy.

Freshly collected plants were used for electron microscopy where possible. Primary fixation was carried out in the field or immediately

upon arrival at the laboratory. Material was fixed in a solution of 25% glutaraldehyde (v/v), .15M phosphate buffer at pH 7.2 and sterilized sea water (1:2:2) for 1 hr., washed and followed by postfixation in a mixture of 2% osmium tetroxide (v/v) and phosphate buffer at pH 7.2 (1:1) for 1½ hr. Fixation and postfixation were carried out at 4°C or at room temperature. After a thorough washing in buffer, the material was then subjected to dehydration in a graded ethanol series, infiltration in propylene oxide and subsequent embedding in Epon 812 (Luft, 1961) or Maraglas 655 (Spurlock, Kattine and Freeman, 1963). Alternatively, the material was embedded in Spurr's medium (Spurr, 1969) directly after the ethanol series.

Thin sections were cut using glass knives or a Du Pont diamond knife on a L.K.B. Ultratome I. Poststaining was carried out for 20-30 min. in uranyl acetate (2% sol. (w/v) in 50% methanol (v/v)) and for 5-10 min. in lead citrate (Reynolds, 1963). The prepared material was examined using a Hitachi HU 11A electron microscope or an AEI 801 A electron microscope operating with an accelerating voltage of 50 KV.

Light microscopy was done with living material using a Wild M20 light microscope. When transverse sections were required, specimens were prepared with a freezing microtome.

III. DISTRIBUTION, LIGHT MICROSCOPIC MORPHOLOGY AND LIFE HISTORY

a) Smithora

Introduction.

Smithora naiadum was first described as a member of the genus Porphyra (Anderson, in Blankinship and Keeler, 1892). The initial definitive account of the morphology of this alga was given by Hus (1903):

"Fronds 2-10 cm. long, obovate when young, oblanceolate when older; base cushion-shaped; fronds wine-red to blue-purple; monostromatic vegetative part 25-30 microns thick, cells square or slightly higher than broad, 15-20 microns high; surface jelly measuring about 5 microns, little jelly between the cells; fronds dioecious ?; sporocarps with 8 carpospores."

The author describes these latter structures as arising in the terminal parts of the blade. The "sporocarp" (carposporangium) gives rise to eight carpospores (two layers of four) which are released. Since this report there have been no other convincing accounts of these structures.

Knox (1926) carried out more extensive investigations on "Porphyra" naiadum and correctly interpreted the pattern of asexual reproduction in describing the development of mature plants from monospores. She made numerous unsuccessful attempts to culture these reproductive units. Her lack of success appeared to be due to poor culture facilities. In addition, Knox claimed to have observed external sexual fusion, antheridial areas and cell division but her figures and descriptions are inconclusive.

At a later date, a morphological re-evaluation carried out by Hollenberg (1959) resulted in the placement of this alga in a new genus, Smithora, and a new family, Erythropeltidaceae. Subsequently, this action has been given biochemical support by Rees and Conway (1962). Hollenberg's description of the plant is as follows:

"Plants epiphytic, with numerous obovate to cuneate and monostromatic blades arising from a prostrate cushion-like perennial multistratose

base; cells with a single stellate chromatophore; plants with no rhizoidal processes arising from the lower cells of the blades; carpospores formed in irregular, mostly terminal sori, in packets of eight; spermatangia arising in irregular sori toward the middle portions of the blades as small cells cut off externally from colored cells of the locally distromatic portions of the blades; plants reproducing asexually by means of irregular, terminal, monostromatic gelatinous sori which are released as a unit."

The account of carpospore formation is essentially based on Hus' description. Hollenberg states: "...it is practically impossible to distinguish such reproductive areas from spermatangial areas...". Hollenberg also reported the presence of additional asexual reproductive units termed "neutral spores" which are formed at the margins of blades and a correlation of monospore release with periods of low tides. Like Knox (1926), he was largely unsuccessful in culturing the spores.

Most recently, Richardson and Dixon (1969) have detailed the presence of a filamentous "conchocelis" stage in the life cycle of Smithora. However, the authors observed none of the reproductive structures described above and included no light micrograph of their findings.

Observations and Discussion.

Distribution: The previously recorded distribution of Smithora naiadum is from northern British Columbia to Isla Magdalena, Baja California, Mexico (Dawson, 1961). However, from collections by various workers, the following Alaskan specimens are recorded in the phycological herbarium at the University of British Columbia (WS indicates wet stack):

Barrier Is.: UBC 22153, 23467-23468, 1.VII.1965. Cape Bartolome: UBC 23466, 2.VII.1965. Cape Chiniak, Kodiak Is.: UBC 9912-9913, 23.VI.1960. Klokachef Is.: UBC 22627-22628, 30.VI.1965. Pasagashak Pt., Kodiak Is.: UBC 8846, 25.VI.1960. Pt. Alexander, Wrangel Narrows: UBC 23469, 1.VII.1965.

Thus, the revised North American Pacific coast distribution for Smithora naiadum is from Kodiak Island, Alaska to Isla Magdalena, Baja California, Mexico.

BRITISH COLUMBIAN COLLECTION RECORDS.

WEST COAST VANCOUVER ISLAND: Amphitrite Pt., Uclulet: UBC 39967, 8.V. 1969; UBC 40146, 18.V.1969; UBC 40248-40249, 1.VII.1969. Bamfield: UBC 40794, 3.VI.1969; UBC 41012, 17.VI.1969; UBC 41154, 9.VII.1969; UBC 41588, 14.VII.1969; UBC 42084, 12.VIII.1969; UBC 42445, 25.VII.1969. Black R.: UBC 13200, 8.VII.1959. Brooks Pen.: UBC 36269, 13.VIII.1968. Bunsby Is.: UBC 19668, 6.IX.1964. Cape Scott: UBC 35769, 36581, 36977, 11.VIII.1969. Cape Sutil: UBC 15741, 15879-15882, 2.VII.1962. Experiment Bight: UBC 19353, 7.VIII.1964. Fisherman Bay: UBC 38295, 38302-38303, 30.VIII.1968. Garden Is.: UBC 10925-10927, 11006, 27.V.1959. Guise Bay: UBC 19770, 7.VIII.1964. Macquinna Pt.: UBC 11258, 24.V.1959. Mills Pen.: UBC 10920-10921, 13314-13315, 7.VII.1959. Nootka Is.: UBC 37512, 22.VIII.1968; UBC 37168, 37679, 37687, 24.VIII.1968. Perez Rock: UBC 10922-10923, 13524, 24.VI.1959. Point No Point (Glacier Pt.): UBC 18759, 2.IX.1968; UBC 18760, 4.VIII.1968; UBC 18761, 7.VI.1968; UBC 38795, 38802, 28.XI.1968; UBC 42359, 16.V.1969; UBC 39011, 39022, 25.X.1968; UBC 39177, 22.XI.1968; UBC 837 WS, 22.XI.1968. San Josef Bay: UBC 38614-38615, 39023, 31.VIII. 1968. Spring Is.: UBC 13525, 25.VII.1959; UBC 18985, 6.VI.1964. Tofino: UBC 39334, 3.V.1969. Topknot Pt.: UBC 42817, 2.IX.1968. Whiffen spit: UBC 38796, 28.IX.1968; UBC 39010, 25.X.1968; UBC 39185, 39329, 22.XI.1968; UBC 46085, 8.XII.1971. Winter Harbour: UBC 11021, 31.V.1959. Wouwer Is.: UBC 10924, 11256, 21.V.1959. Yellow Bluff: UBC 37282, 37505, 37511, 21.VIII. 1968; UBC 915 WS, 21.VIII.1968.

EAST COAST VANCOUVER ISLAND: Cluxewe R.: UBC 17075, 5.VII.1962. Deer Is.: UBC 35576-35577, 9.VIII.1968. Discovery Is.: UBC 38797, 3.IX.1968. False Head: UBC 38617-38618, 4.IX.1968. Gabriola Is.: UBC 28375, 30.III.1967; UBC 28826, 28.IV.1967; UBC 29574, 29609, 24.V.1967. Grassy Is.: UBC 36056, 37276, 38613, 20.VIII.1968. Quadra Is. (south): UBC 43793, 10.IX.1970.

SCOTT ISLAND GROUP: Cox Is.: UBC 36055, 36592, 36752, 12.VIII.1968. Triangle Is.: UBC 19578, 7.IX.1964.

MAINLAND (JOHNSON STRAIT AREA): Neville Pt.: UBC 15963, 29.VI.1962.

PRINCE RUPERT AREA: Chetham Channel: UBC 15110-15111, 8.VII.1962. Digby Is.: UBC 16450, 26.VI.1963.

QUEEN CHARLOTTE ISLANDS: Anthony Is.: UBC 16551, 16554, 19.VI.1963. Marchant Reef, Graham Is.: UBC 21343, 22301, 4.VII.1965. Striae Is.: UBC 16240, 16282, 24.VI.1963.

Field Material: Following examination of many of the above herbarium specimens and numerous collections during the period from September, 1968 to November, 1971, I am in general agreement with Hollenberg's (1959)

results. However, no convincing evidence of carpospores or neutral spores was obtained.

There was a marked difference among winter populations of Smithora in different areas. At the collecting sites on Vancouver Island (Sooke and Point No Point) the basal cushions remained throughout the winter, but in Stanley Park all traces of the plant disappeared in mid-November only to become established again the following spring. The latter collecting area has a lower salinity (near a freshwater outflow) and is much more protected than the other sites. If Richardson and Dixon's conchocelis phase exists, perhaps it serves as an overwintering mechanism in certain populations.

"Spermatangia" as described by Hollenberg (1959) were observed regularly in the fall months of each year. However, there was no convincing indication of fertilization taking place or having taken place. Indeed, there is no good evidence implicating "spermatia" in sexual fusion in any of the Bangiophycidae. Although they will be referred to as "spermatangia/spermatia" in this report, perhaps they may be likened to the β -spores of Porphyra (Conway, 1964) which are formed in a different manner. These structures will be discussed further in Section VI.

Cultured Material: Limited success was obtained in culturing Smithora. Sterile blades could be cultured for periods up to four months. Monosporic blades would not survive beyond one month, although during this period they would continue to differentiate and release monospores. These structures are usually released terminally but, in culture, isolated patches of precociously released spores could be observed toward the center of the sorus (Pl. III, Fig. 6). It appears that artificial conditions somewhat disrupt the synchrony of spore production. Monospores would germinate readily, either singly or in masses, to form basal cushions (Pl. III, Fig. 1). These cushions

would then produce single monospores (Pl. III, Fig. 1,2,3) which were identical to those produced by the blade. After release, the spore would divide (Pl. III, Fig. 4) to produce a new basal cushion (Pl. III, Fig. 5). Such cushions showed no evidence of blade formation although several generations could be maintained in culture. Another characteristic of these structures to which Knox (1926) briefly alluded is their ability to adhere to one another (Pl. III, Fig. 5).

Culture of spermatangial blades proved unfruitful, although a good release of these cells could be obtained. The presently known life history of Smithora is diagrammatically illustrated in Pl. IV.

Recently, Harlin (1971) has shown that Smithora will grow in the field on polyethylene strips approximating the dimension of Phyllospadix leaves. Present results on cultured material in this laboratory also appear to indicate that this alga is not dependent on its host for any nutritive material as was thought by some authors (e.g. Knox, 1926). However, it suggests that a delicate set of environmental conditions is needed for the plant to produce the leafy thallus. Adjustments in culture parameters such as ingredients of the media, temperature, agitation, light intensity and light duration had no effect.

PLATE II.

Map of British Columbia showing collection sites of Smithora naiadum,
Erythrotrichia carnea, Erythrotrichia boryana and Erythrotrichia pulvinata
as recorded in the phycological herbarium of the University of British
Columbia by various workers.

Legend.

| | | | |
|-----------------------|-----|-----------------------------|-----|
| Acous Peninsula..... | 23. | Lawton Pt..... | 37. |
| Amphitrite Pt..... | 31. | Lippy Pt..... | 19. |
| Anthony Is..... | 5. | Lookout Is..... | 32. |
| Bamfield..... | 35. | Macquinna Pt..... | 28. |
| Black River..... | 38. | Marchant Reef..... | 3. |
| Brooks Peninsula..... | 22. | Mills Peninsula..... | 36. |
| Bunsby Is..... | 7. | Neville Pt..... | 46. |
| Cape Scott..... | 13. | Nootka Is..... | 27. |
| Cape Sutil..... | 10. | Perez Rock..... | 29. |
| Chatham Channel..... | 1. | Piper's Lagoon..... | 44. |
| Cluxewe River..... | 47. | Plover Is..... | 7. |
| Cox Is..... | 15. | Point No Point..... | 39. |
| Deer Is..... | 49. | Qlawdzeet-Bell Passage..... | 9. |
| Digby Is..... | 2. | Quadra Is..... | 45. |
| Discovery Is..... | 41. | San Josef Bay..... | 17. |
| Experiment Bight..... | 12. | Stanley Park..... | 42. |
| False Head..... | 48. | Striae Is..... | 4. |
| Fisherman Bay..... | 11. | Tofino..... | 30. |
| Gabriola Is..... | 43. | Topknot Pt..... | 18. |
| Garden Is..... | 33. | Triangle Is..... | 14. |
| Grassy Is..... | 25. | Whiffen spit..... | 40. |
| Guise Bay..... | 16. | Winter Harbour..... | 20. |
| Hedley Is..... | 8. | Wouwer Is..... | 34. |
| Hope Is..... | 6. | Yellow Bluff..... | 26. |
| Lawn Pt..... | 21. | | |

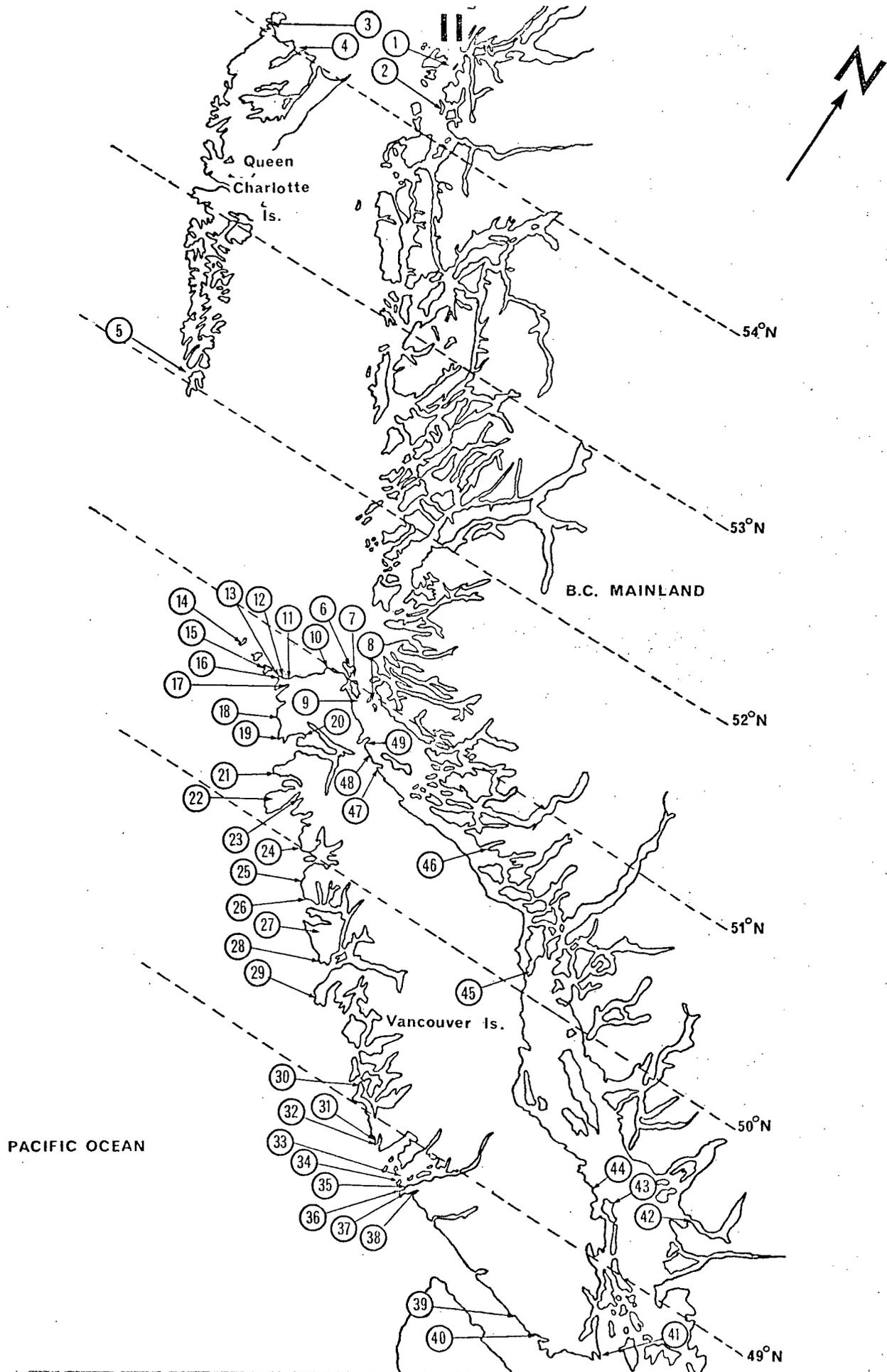


PLATE III.

Light micrographs. Smithora

Fig. 1. Basal holdfast (bh) in culture producing second generation monospores (arrow).

Fig. 2. Differentiating monospore in cultured first generation basal pad. vc denotes vegetative cell.

Fig. 3. Second generation monospore in culture.

Fig. 4. 2-celled stage of germinating monospore in culture.

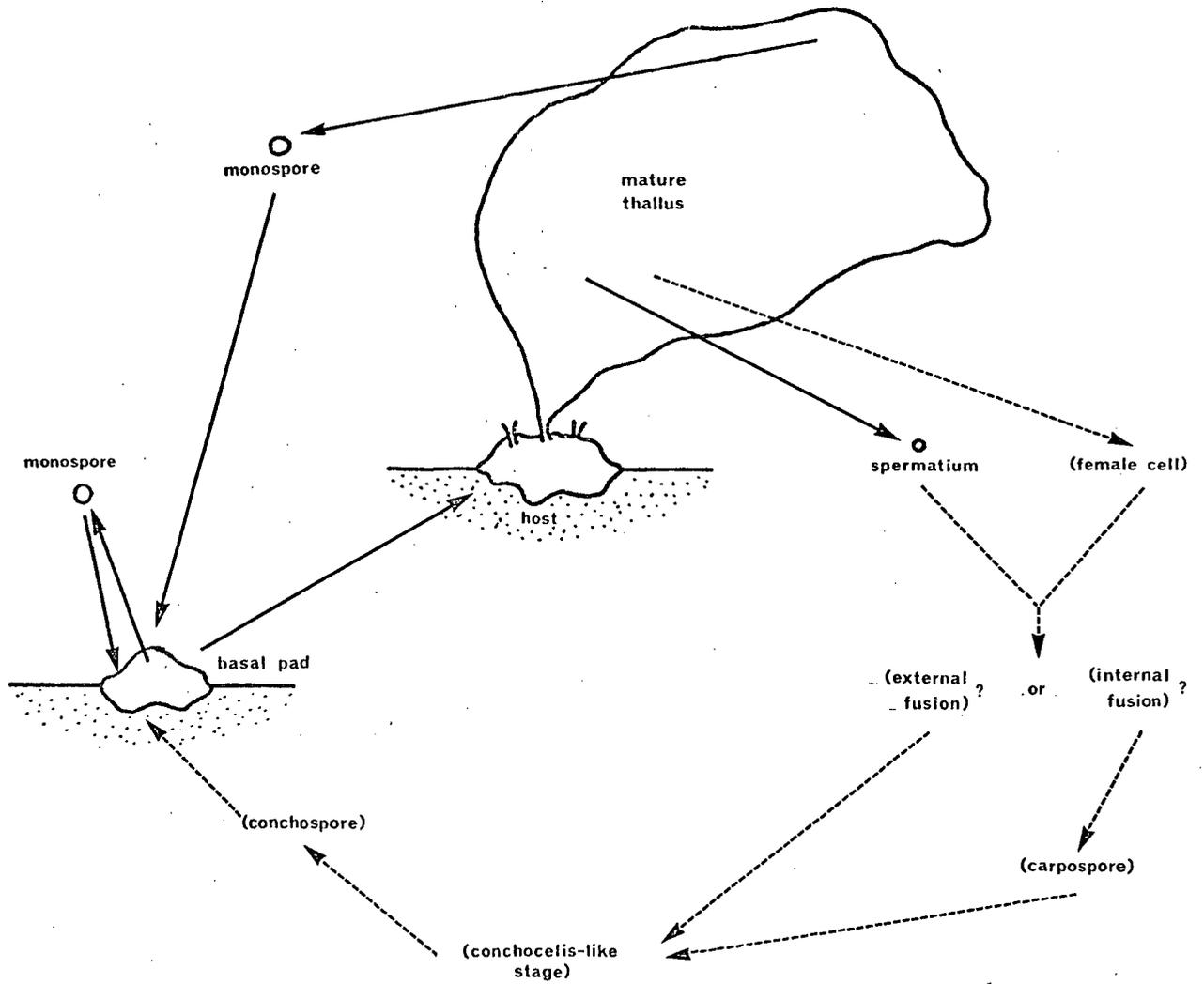
Fig. 5. Young cultured basal holdfast.

Fig. 6. Portion of mature blade showing differentiating monospores (dm), vegetative cell area (vc) and an isolated area of precociously released monospores (rm).

PLATE IV.

Diagram of the possible life cycle of Smithora naiadum. Dotted lines indicate poorly documented steps of the scheme.

IV



b) Erythrotrichia

Introduction.

Some 36 species of Erythrotrichia Areschoug have been described from various parts of the world. Because of this wide distribution, an examination of each recorded species or growth form would be, at best, extremely difficult. This situation has resulted in taxonomic confusion and uncertainty.

Historically, the following characteristics have been used to determine species of Erythrotrichia: colour, size, form of filament (monosiphonous, polysiphonous, ribbon-shaped, etc.), type of chloroplast (parietal or stellate), branching and host specificity. Numerous attempts have been made to assign some type of natural system of classification to these plants. Berthold (1882) divided the genus into two groups: one forming spores which directly give rise to filaments, the other forming spores which give rise to basal discs, followed by the secondary process of filament formation. Hamel (1929) distinguished three categories: those attached by a single, lobed or unlobed basal cell, those attached by a number of rhizoidal cells and those attached by a multicellular disc. Tanaka (1952) proposed a taxonomic bisection on the basis of parietal or stellate chloroplasts. More recently, Heerebout (1968) has recommended the recognition of only three species, due to the morphological variability of these plants in culture. Unfortunately, he was unable to examine representatives of all described species and rejected many only on the basis of published reports. His description of the genus and key to the species are as follows:

"Erythrotrichia. Thallus erect, filamentous or ribbon-shaped, often with a disc or cushion-shaped attachment organ. Filamentous thallus branched or unbranched, mono- or polysiphonous. Ribbon-shaped thallus always monostromatic; unbranched. Cells brick red, length about 10-25 microns. Chromatophore stellate with a distinct pyrenoid. Asexual reproduction by monospores; life cycle with a conchocelis stage. Pit connections never seen.

Key to the species.

- 1a. Thallus consisting of rows of cells arranged in one plane, giving it a ribbon-shaped appearance. Nearly always with a basal disc, in young stages sometimes only a basal disc is present.....E. boryana.
- 1b. Thallus mono- or polysiphonous, cell rows radially arranged...2.
- 2a. Thallus often attached by a basal disc or by small protuberances of the basal cell.....E. carnea.
- 2b. Thallus with a long boring rhizoid, composed of hyaline cells, always growing on Ralfsia thalli attached to gastropods...
.....E. welwitschii."

Heerebout considered such characteristics as chloroplast morphology, mono- or polysiphony and mode of attachment to be unreliable for taxonomic purposes. West (1966) has also questioned the validity of using certain taxonomic criteria. In contrast, Dangeard (1968,1969) lists 34 species and colourfully describes Heerebout's revision as follows: "...sans doute en premiere dans ce 'massacre' d'espèces...".

Details of certain reproductive processes in Erythrotrichia are equally obscure. Asexual reproduction is accomplished primarily through monosporogenesis, whereby a vegetative cell undergoes a division and one of the resulting daughter cells forms a spore. In one species (E. welwitschii) an undivided vegetative cell may be released as a unit.

Reports of sexual reproduction have been sporadic. Berthold (1882) first described spermatia being cut off from a vegetative cell, released and attaching to a filament adjacent to the supposed carpogonium. Subsequent reports of such events by Gardner (1927), Baardseth(1941) and Tanaka (1944, 1952) have shed little additional light on this process. For example, concerning post-fertilization events, Berthold (1882) describes an undivided, fertilized carpogonium being released whereas Tanaka (1944) states that fertilized carpogonia divide to produce a "few" carpospores. Heerebout (1968)

has reported the presence of a conchocelis phase of the life cycle of Erythrotrichia which he presumes to have grown from carpospores, although no direct evidence of this is presented.

Thus, it is obvious that there is a need of critical research in almost every phycological aspect of this genus.

Observations and Discussion.

Pacific Coast Distribution¹: Three species of the genus Erythrotrichia are reported here according to the revision proposed by Heerebout (1968), with the exception that E. pulvinata has been retained as a valid species. The distinguishing morphological feature is the presence of a relatively large, monospore producing, multistromatic, basal holdfast. In addition, E. pulvinata appears to occupy a specialized habitat (epiphytic on the utricles of Codium fragile). In light of these findings, a re-examination of specimens of Erythrotrichia reported by various authors on the Pacific coast of North America is required in order to obtain a more complete distributional record of this taxon.

Erythrotrichia boryana

The previously recorded distribution is from Punta Baja to Bahía Asunción, Baja California, Mexico (Dawson, 1961).

BRITISH COLUMBIAN COLLECTION RECORDS.

WEST COAST VANCOUVER ISLAND: Point No Point (Glacier Pt.): UBC 1280 WS, 2.VII.1971 (epiphytic on Phyllospadix scouleri and Smithora naiadum).

Erythrotrichia carnea

The previously recorded distribution is from Monterey, California to Golfo Dulce, Costa Rica; Clipperton Is. (Dawson, 1961) with a northward

¹This part of the thesis is based on an article by J.W. Markham, D.L. McBride and P.R. Newroth which has been accepted for publication in Syesis.

extension by Norris and West (1967) at Shilshole Bay Marina, Seattle, Washington.

BRITISH COLUMBIAN COLLECTION RECORDS.

WEST COAST VANCOUVER ISLAND: Bamfield: UBC 648 WS, 30.VII.1969 (epiphytic on Callithamnion pikeanum). Garden Is.: UBC 964 WS, 27.V.1959 (epiphytic on Polysiphonia pacifica); UBC 972 WS, 27.V.1959 (epiphytic on Corallina vancouveriensis). Point No Point (Glacier Pt.) UBC 1260 WS, 8.VII.1971 (epiphytic on Codium fragile).

EAST COAST VANCOUVER ISLAND: Piper's Lagoon: UBC 895 WS, 2.VIII.1959 (epiphytic on Lomentaria sp.).

Erythrotrichia pulvinata

The previously recorded distribution is from Middle Bay, Oregon to Bahía Asuncion, Baja California, Mexico (Dawson, 1961).

BRITISH COLUMBIAN COLLECTION RECORDS.

WEST COAST VANCOUVER ISLAND: Bamfield: UBC 41754, 28.VII.1969; UBC 41671, 647 WS, 29.VII.1969 (epiphytic on Codium fragile). Point No Point (Glacier Pt.): UBC 1259 WS, 6.X.1971 (epiphytic on Codium fragile).

Field and Cultured Material: Both E. carnea and E. boryana were typical in appearance (Pl. V, Fig. 7,11), readily differentiating and releasing monospores in culture. Upon germination, the monospores of E. carnea exhibit a polarity (Pl. V, Fig. 8), forming one or two erect filaments (Pl. V, Fig. 9). In E. boryana monospores divide to produce a simple monostromatic basal disc (Pl. V, Fig. 12) from which filaments are derived (Pl. V, Fig. 11). E. carnea regularly exhibited in situ monospore germination to give an appearance of branching (Pl. V., Fig. 10) as described by Dixon and West (1967). E. boryana may also be attached to Smithora. (Pl. V, Fig. 13). Structures which could be interpreted as spermatia and carpospores were observed in freshly collected E. boryana and will be discussed at length in Section VI.

Very few studies have been carried out on E. pulvinata. In his original description Gardner (1927) states that the pad may be found without the filamentous thallus. In the population used for this study, I have observed

filamentous thalli only once (Pl. VI, Fig. 14), although the basal cushions appear to thrive. As was suggested in the discussion on Smithora (Section III), a delicate set of environmental conditions may be required for blade formation and perhaps this relatively exposed site (Point No Point) does not provide them.

Dawson (1953) described monospore production in E. pulvinata and Hollenberg (1971) reasserted the fact that they are formed in the usual manner. Results in this laboratory indicate that, as in Smithora, the basal cushion is also capable of producing these reproductive structures (Pl. VI, Fig. 15). Upon germination in culture, they form a cell wall and divide (Pl. VI, Fig. 16,17) to produce a large, multicellular holdfast (Pl. VI, Fig. 18). The cells at the edge of the pad are elongated, possibly due to a greater rate of division (Pl. VI, Fig. 19). Evidence of additional reproductive structures and filament formation was not obtained.



PLATE V.

ERYTHROTRICHIA

- Fig. 7. E. carnea. Mature filaments producing monospores (arrow) in culture.
- Fig. 8. E. carnea. Bipolar germination of cultured monospores.
- Fig. 9. E. carnea. Juvenile filaments developing from one monospore in culture ("tripolar" germination).
- Fig. 10. E. carnea. In situ germination of cultured monospores.
- Fig. 11. E. boryana. Mature filaments and basal disc.
- Fig. 12. E. boryana. Cultured basal pad.
- Fig. 13. E. boryana. Freezing microtome section of association between E. boryana and Smithora (h).

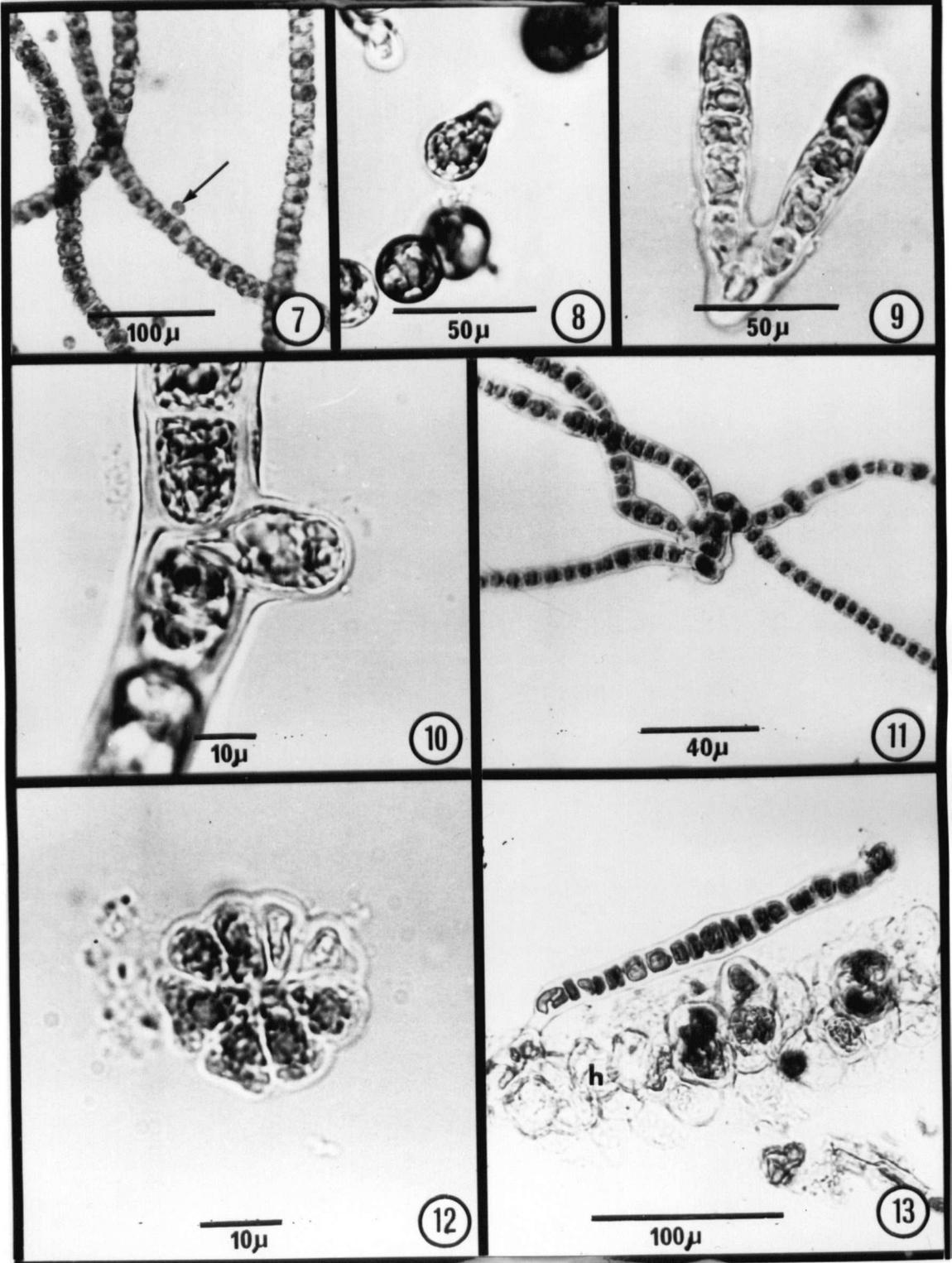


PLATE VI.

Erythrotrichia pulvinata

Fig. 14. Portion of mature filament.

Fig. 15. Monospore production (arrow) from basal holdfast (bh) in culture.

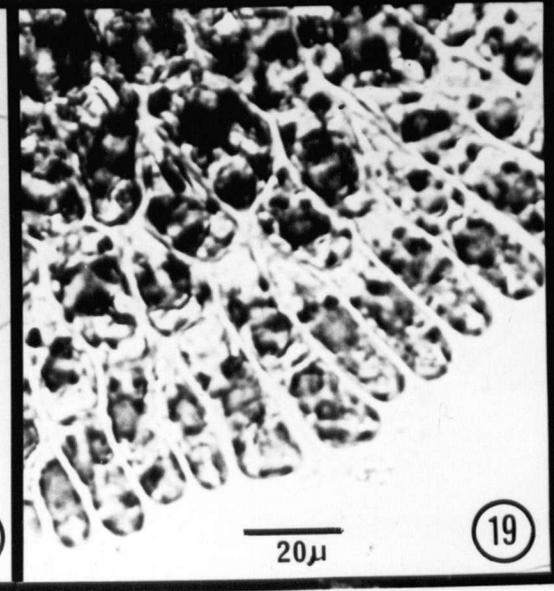
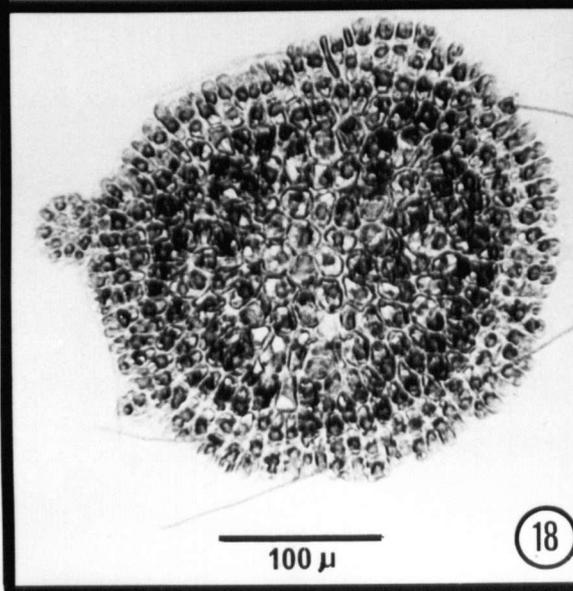
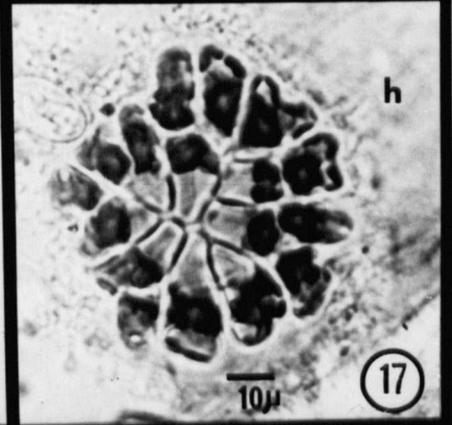
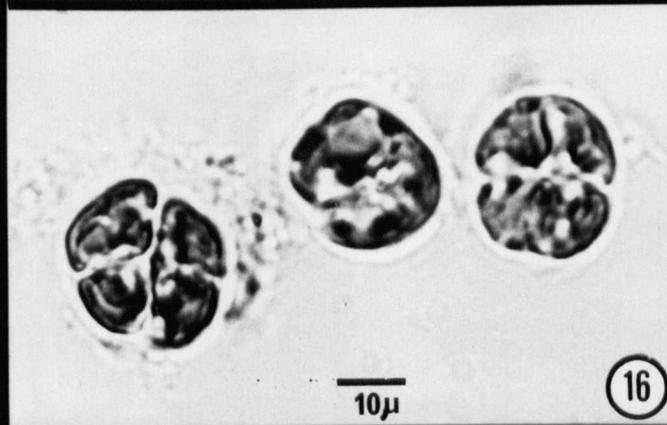
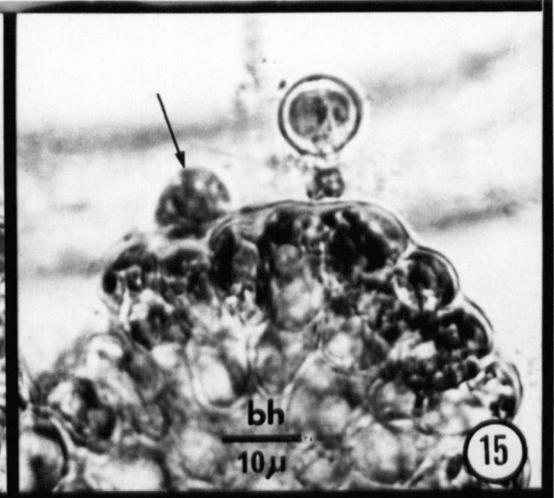
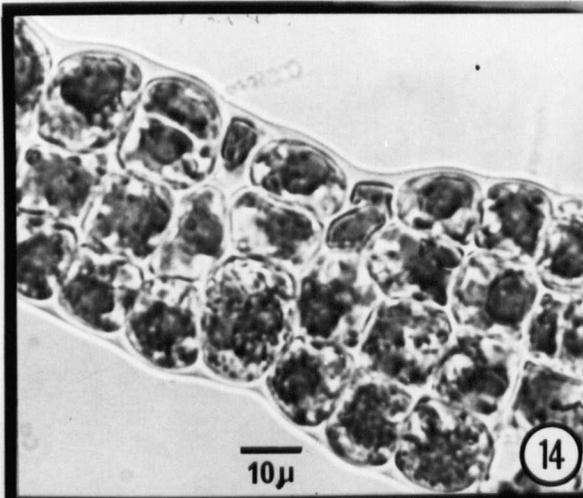
Fig. 16. 4-celled stage of cultured, germinating monospore.

Fig. 17. Juvenile pad attached to utricle of Codium fragile (h).

Fig. 18. Mature pad growing in culture on glass Petri dish.

Fig. 19. Edge of cultured pad showing elongate cellular shapes.

VI



IV. ULTRASTRUCTURE OF THE VEGETATIVE CELL

Introduction.

Recently there has been a growing interest in ultrastructural details concerning members of the Rhodophyceae. Much of the research has been done with the larger and "more advanced" subclass Florideophycidae. There appear to be published reports describing six genera of the "less advanced" Bangiophycidae. Of these, Porphyridium, a unicellular form, has drawn much attention (Brody and Vatter, 1959; Speer, Dougherty and Jones, 1964; Gantt and Conti, 1965, 1966; Gantt, Edwards and Conti, 1968; Guerin-Dumartrait, Sarda and Lacourly, 1970; Neushul, 1970; Wehrmeyer, 1971; Chapman, Chapman and Lang, 1971; Ramus, 1972). Porphyra has also been investigated by a series of authors (Gibbs, 1960; Ueda, 1961; Yokomura, 1967; Kito and Akiyama, 1968; Kazama and Fuller, 1970; Bourne, Conway and Cole, 1970; Loe and Fultz, 1970; Bourne, 1971; Cole, 1972). Evans (1970) described a new genus, Rhodella, primarily on the basis of electron microscopy. A certain amount of information is also available on Bangia (Honsell, 1963; Sommerfield and Leeper, 1970), Rhodosorus (Giraud, 1963) and Compsopogon, a freshwater form (Nichols, Ridgway and Bold, 1966). To my knowledge there are no published ultrastructural accounts dealing with members of the Erythropeltidaceae.

a) Smithora¹

Observations.

Vegetative cells of Smithora are approximately 10 microns in diameter although some monostromatic areas of the thallus may contain larger cells.

¹This portion of the thesis is based on a publication by D.L. McBride and K. Cole in Phycologia 8, 177-186 (1969). The text of the original article has been brought up to date by including subsequent references where appropriate.

Each consists of a thick cell wall and an irregular protoplast with a large chloroplast, mitochondria, endoplasmic reticulum, dictyosomes and a single nucleus (Pl. VII, Fig. 2). Additional structures within the cell include floridean starch granules, vacuoles and many multivesicular bodies.

The cell wall, composed of two or three distinct layers, appears to be similar to that of Porphyra (Frei and Preston, 1964; Bourne, 1971) (Pl. VII, Fig. 2; Pl. VIII, Fig. 5). According to Frei and Preston (1964), the fibrous organization of these cell wall layers is due to microfibrils composed of β -1,3 linked xylans. The layering effect seems to be due to a different organization of the microfibrils with the outer layers being more compacted. A nonfibrillar outermost layer which could be analagous to the mannan-containing cuticle of Porphyra (Frei and Preston, 1964; Hanic and Craigie, 1969) was also noted in Smithora.

The shape of the protoplast is much more variable than any previously reported in the red algae. Pseudopodia-like extensions and invaginations are evident in most cells (Pl. VII, Fig. 2). Wall material seems to be isolated within the cell when the invaginations are viewed in cross-section.

The single, lobed chloroplast, bounded by a double membrane, occupies most of the cell (Pl. VII, Fig. 2; Pl. VIII, Fig. 5). In some sections the lobes may appear as separate entities isolated from the main body of the chloroplast (Pl. VII, Fig. 2; Pl. VIII, Fig. 3). The arrangement of chloroplast lamellae in younger cells is very regular, each individual thylakoid being orientated parallel to the others (Pl. VII, Fig. 2). There is one thylakoid which follows the contour of the chloroplast envelope (peripheral thylakoid). However, at no time was the chloroplast envelope continuous with any of the thylakoids. The same general chloroplast structure is also characteristic of older cells but the lamellae do not appear as

smooth or as regularly parallel (Pl. VIII, Fig. 5).

Thylakoid associations such as those reported in other algal groups (Kirk and Tilney-Bassett, 1967) have not been noted previously in the Rhodophyta. Thus, a most interesting feature of the chloroplast of Smithora is a stacked arrangement of varying numbers of lamellae in certain restricted areas (Pl. VII, Fig. 2; Pl. IX, Fig. 6,7). The lamellar stacks are almost exclusively formed by an overlapping of thylakoid edges. This results in a narrow, localized stack usually situated near the pyrenoid. Fusion and forking of lamellar edges often occur in these areas.

Intermittent fusion of photosynthetic lamellae is found throughout the chloroplast of Smithora (Pl. VIII, Fig. 5). However, this lamellar fusion rarely occurs in the regular arrangement seen in Porphyridium (Gantt and Conti, 1965). Lamellar spirals similar to those reported in Porphyridium (Gantt and Conti, 1965) were also noted in Smithora (Pl. VIII, Fig. 3).

The centrally located pyrenoid is similar to that reported in other red algal species (Gibbs, 1962a). It is often penetrated by lamellae which appear swollen and frequently form common vesicles within its matrix (Pl. VII, Fig. 2; Pl. VIII, Fig. 5; Pl. IX, Fig. 7).

Numerous electron transparent areas, sometimes containing a fibrillar material, are scattered throughout the chloroplast between the thylakoids (Pl. VII, Fig. 2; Pl. VIII, Fig. 5). These structures have been interpreted as localized areas of DNA and have been found in other red algal species, e.g. Laurencia (Bisalputra and Bisalputra, 1967) and Porphyra (Yokomura, 1967). Osmiophilic droplets are also frequently observed between the lamellae (Pl. VII, Fig. 2; Pl. VIII, Fig. 5). No convincing evidence indicating the presence of phycobilisomes has been found although these structures

could have been lost during preparation of the specimens.

Typical red algal floridean starch occurs within the cytoplasm in the form of ellipsoidal granules (Pl. VII, Fig. 2; Pl. VIII, Fig. 5). These granules vary in staining intensity in a manner similar to that reported in Porphyridium (Gantt and Conti, 1965).

Mitochondria with tubular cristae are numerous and variable in size and shape (Pl. VII, Fig. 2; Pl. IX, Fig. 8; Pl. X, Fig. 10). Infrequently, a mitochondrion in a "doughnut" or ring formation was noted (Pl. IX, Fig. 8). Other structures often occur within the centre of these atypical mitochondria. No extremely long or branched mitochondria similar to those found in Porphyridium (Gantt and Conti, 1965) were observed in Smithora.

Both rough and smooth endoplasmic reticulum occur in these cells, usually following the contour of the plasmalemma or the nuclear envelope. (Pl. VII, Fig. 2; Pl. VIII, Fig. 4; Pl. X, Fig. 10). However, no connections between these entities have been found. In addition, what appears to be more densely staining ER is seen intermittently in the protoplast extensions (Pl. IX, Fig. 8). One or more dictyosomes are often found in a single cellular cross-section (Pl. VII, Fig. 2). They consist of the usual flattened cisternae and associated vesicles. No particular location or function can be assigned to these organelles in the vegetative cell. Younger vegetative cells contain few well defined vacuoles. However, in older cells these structures seem to increase in size and number (Pl. VIII, Fig. 5; Pl. X, Fig. 13).

The nucleus is typically eucaryotic (Pl. VII, Fig. 2; Pl. VIII, Fig. 4). The evenly granular nucleoplasm and densely staining nucleolus are surrounded by a porous nuclear envelope. The nucleolus often occupies a peripheral position in the nucleus of Smithora. However, this structure is not necessarily orientated toward the chloroplast as reported in Porphyridium (Gantt and Conti,

1965).

Lomasome-like bodies were frequently observed in Smithora as membrane bound aggregations of vesicles within the cytoplasm (Pl. X, Fig. 10,12) and as groups of vesicles being released into the cell wall (Pl. X, Fig. 10,11). The vesicles themselves vary in size and are bounded by a single membrane. Marchant and Robards (1968) suggest that those multivesicular bodies in plants which seem to originate from the plasmalemma should be termed plasmalemmasomes and those from within the cytoplasm, lomasomes. Plasmalemmasomes are usually associated with tubular vesicles in Smithora (Pl. X, Fig.11), while the lomasomes seem to be composed mainly of aggregations of spherical vesicles (Pl. X, Fig. 10,12). However, the distinction between these two types of multivesicular bodies is often not clear. Lomasome-like structures have been reported previously in other red algae, e.g. Lomentaria (Bouck, 1962), Laurencia (Bisalputra et al., 1967) and Pseudogloioophloea (Ramus, 1969). In addition, single vesicular structures were frequently noted in the cell wall near the plasmalemma (Pl. X, Fig. 13). Similar structures were described in Laurencia (Bisalputra et al., 1967) and the green alga Chara (Barton, 1965). In older cells various types of whorled lamellar bodies were seen as well (Pl. X, Fig. 13). These have also been noted in older cells of Porphyridium (Gantt and Conti, 1965), Polysiphonia (Rawlence and Taylor, 1972) and Batrachospermum (Brown and Weier, 1970).

Sections were made at the junction of the basal portion of Smithora and the host tissue, which yielded no evidence of cytoplasmic connections between the individual cells of the host and the epiphyte. The respective cell walls seem to be merely cemented together in a smooth plane. In addition, there were no intercellular connections within the alga itself.

Discussion.

From time to time various authors have presented evidence supporting a phylogenetical relationship between the Rhodophyta and other algal groups (Smith, 1955). The theories which have been proposed directly implicate the Bangiophycidae, since this group is thought to possess certain primitive characteristics in common with less advanced algal groups. These include: lack of sexual reproduction in some species, presence of phycobilins and lack of flagella. However, until further research is carried out any such proposal will remain insecure. Ultrastructural studies could be especially valuable in this respect. Indeed, one fine structural characteristic, the presence of unassociated photosynthetic lamellae is considered typical of the red algae (Gibbs, 1960, 1962a). This has been used on occasion as an additional taxonomic character and could support a proposed relationship between the red algae and the Cyanophyta. While Smithora exhibits some of the ultrastructural features considered typical of the Rhodophyceae (thick, layered cell wall and floridean starch stored outside the chloroplast), it is unique thusfar in possessing relatively narrow, loosely associated thylakoid stacks within the chloroplast. These bands appear to be primitive since they are by no means extensive and are almost exclusively restricted to lamellar edges. Because these structures occur randomly in the chloroplasts of older as well as younger cells, they are not believed to be involved in cell division. Frequently one end of an inner thylakoid participates in a lamellar stack while the other end forms or contributes to a swollen vesicle within the pyrenoid. This may indicate that the bands are an integral part of the chloroplast and perform an important function. Consequently, it is evident that other members of the Rhodophyta, in particular the "less advanced" members, should be examined to determine the extent of this

banding phenomenon.

Thus far there seem to be two structural types of red algal chloroplasts depending upon the presence or absence of a pyrenoid. From light microscopic studies it is reported that certain members of the Nemaliales and many of the Bangiophycidae possess pyrenoid-containing chloroplasts (Fritsch, 1945). However, within this group the arrangement of the thylakoids in relation to the chloroplast envelope seems to vary. Smithora displays numerous sheet-like photosynthetic lamellae which tend to parallel the contour of the chloroplast envelope. Some members of the Nemaliales, e.g. Thorea (Bischoff, 1965) and Acrochaetium (McBride, unpubl.) and the unicellular bangiophyte Rhodorus (Giraud, 1963) seem to possess a similar chloroplast structure. Kylinia (Gibbs, 1962a), Nemalion (Gibbs, 1962a, 1962b) and Rhodochorton (Mitrakos, 1960), other members of the Nemaliales, may also possess this feature although published micrographs are inconclusive. In contrast, Porphyra (Bourne, 1971), Porphyridium (Brody and Vatter, 1959 and others), Rhodella (Evans, 1970) and Banqia (Honsell, 1963) have photosynthetic lamellae which terminate at the chloroplast envelope. The significance of this well defined difference in the ultrastructure of red algal, pyrenoid-containing chloroplasts will be discussed in Section VII.

Marchant and Robards (1968) describe two types of multivesicular (paramural) bodies associated with plant cells: lomasomes and plasmalemmasomes. These authors suggest that lomasomes, which have been observed in a large number of plants, may be involved in transport of cell wall precursors across the plasmalemma. They also propose that the plasmalemmasome is concerned with secondary modifications of the cell wall. Various types of these multivesicular bodies have been noted in Smithora. Since formation of a thick supporting cell wall in this alga would undoubtedly entail

important cellular functions, it would seem that this hypothesis is not unreasonable. Ramus (1969) noted an abundance of lomasome-like bodies in Pseudogliophloea associated with the formation of cell wall material between dividing cells.

It is of interest that many more of these structures are observed in older cells of Smithora. Since Smithora's main method of reproduction seems to be vegetative (portions of the monosporic thallus are released periodically) (Hollenberg, 1959), there is a possibility that some of the above mentioned structures may be involved in transport of catabolic enzymes capable of acting on cell wall material. This function would obviously be very important to the plant. Hawker and Gooday (1969) also proposed that lomasomes in the fungus Rhizopus may be associated with cell wall degradation. Since the cells of Smithora contain relatively few vacuoles, another functional possibility of these paramural bodies which could be entertained is the transport of metabolic waste from the cell. In addition, the single vesicles which occur frequently in the cell wall near the plasmalemma may be involved in one or more of the functions discussed here. However, not until cytochemical and autoradiographic techniques progress further can explicit functions be assigned to these various structures.

It is known that certain members of the red algae are parasitic (Fritsch, 1945). The fact that Smithora is an obligate epiphyte tends to arouse one's suspicions that a parasitic relationship may exist. However, no obvious evidence of parasitism was observed in sections through host and epiphytic tissue. Since this alga has a well developed photosynthetic apparatus, this is not unexpected. Nevertheless, it is of interest that Harlin (1971) has reported the possible occurrence of a nutrient transfer in this situation.

There have been several light microscopic reports of pit connections in the Bangiophycidae (see Dixon, 1963 for review). Recent ultrastructural accounts of these structures in the conchocelis phase of Porphyra (Lee and Fultz, 1970; Bourne, Conway and Cole, 1970) and the conchocelis phase of Bangia (Sommerfeld and Leeper, 1970) have conclusively shown that this characteristic can no longer be used to separate the rhodophycean subclasses. However, there is no evidence of pit connections in Smithora. Each cell is a separate entity, no connections of any type remain after division.

PLATE VII.

Smithora

Fig. 2. Cross-section of a typical vegetative cell with a chloroplast (C)¹, chloroplast envelope (CE), pyrenoid (P), mitochondria (M), endoplasmic reticulum (ER), dictyosomes (D), floridean starch granules (FS), cell wall material (CW), DNA pockets (white arrow) and osmiophilic granules (black arrow). Double arrow indicates a lamellar stack.

¹The disparity in the types of notations used to label illustrations in different sections of this report is due to the use of material previously published by the author over a period of time.

VII



PLATE VIII.

Smithora

Fig. 3. Chloroplast lobe exhibiting a spiral arrangement of thylakoids.

Fig. 4. Nucleus consisting of nucleoplasm and a nucleolus (Nu) surrounded by a nuclear envelope (NE).

Fig. 5. Older cell with chloroplast and centrally located pyrenoid containing swollen vesicle-like lamellae (LV). Vacuoles (V) and multi-vesicular bodies (Mv) are typical of older cells. Thylakoid fusion (arrow) and floridean starch granules (FS) are also present.

VIII



PLATE IX.

Smithora

Fig. 6. Thylakoid stack. Arrow indicates periodic branching of lamellae associated with stack formation.

Fig. 7. Thylakoid stack. Note lamellar branching (arrow) and swollen vesicle-like lamellae within the pyrenoid.

Fig. 8. Ring shaped mitochondrion (RM) exhibiting cristae and mitochondrial envelope. Two other mitochondria and a vesicular structure (Ve) are located in the centre. Darkly stained ER-like material (arrows) is also present in an adjacent cytoplasmic lobe.

Fig. 9. Point of junction between algal cell wall (AW) and host cell wall (HW). Part of algal protoplast (Pr) is also shown.

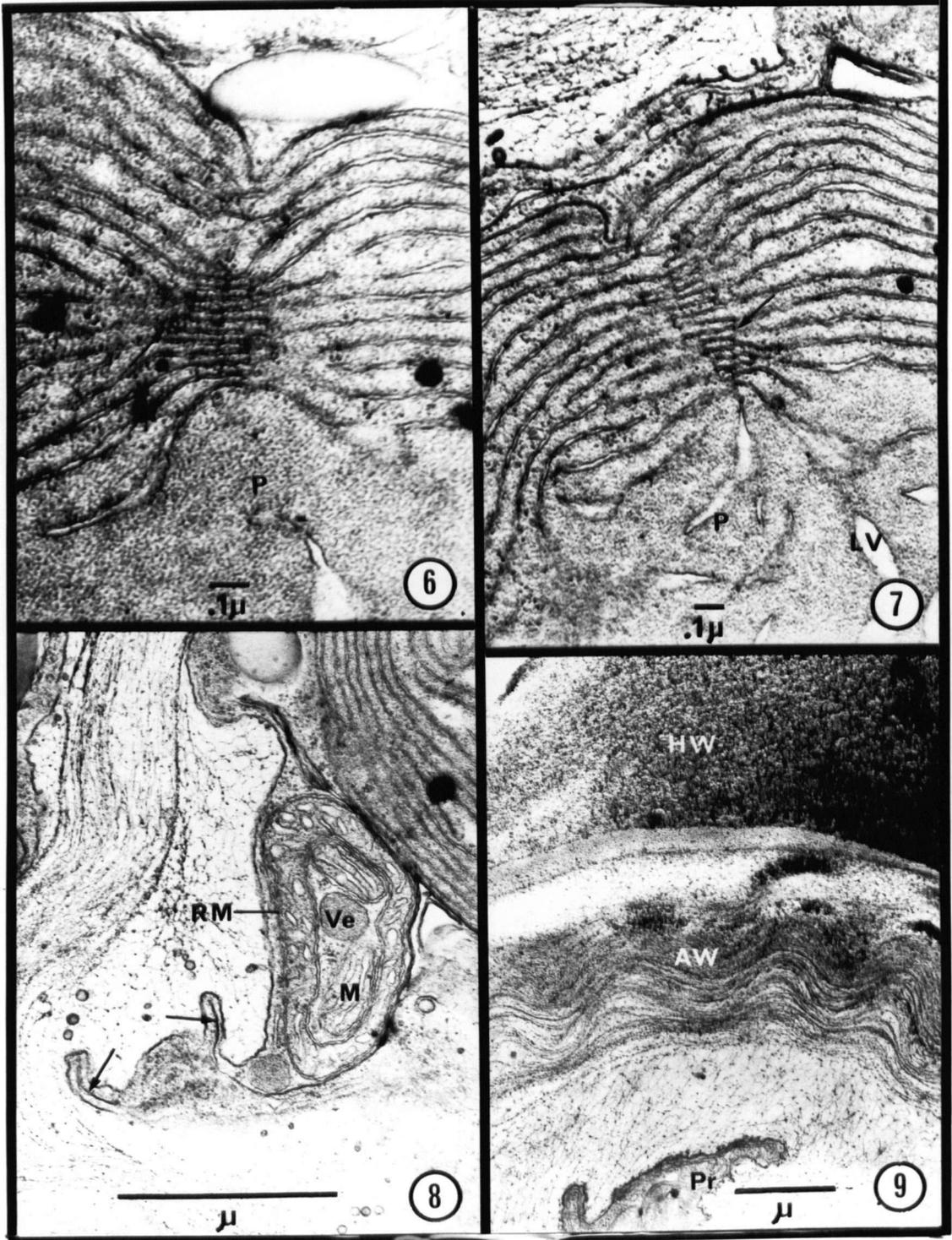


PLATE X.

Smithora

Fig. 10. Section through portions of two neighbouring cells illustrating multivesicular bodies (arrows) consisting mainly of spherical vesicles. Other structures include a mitochondrion, endoplasmic reticulum, chloroplast with lobe (CL) and cell wall material.

Fig. 11. Multivesicular bodies (arrows) consisting of many elongate tubular vesicles associated with the plasmalemma (Pl).

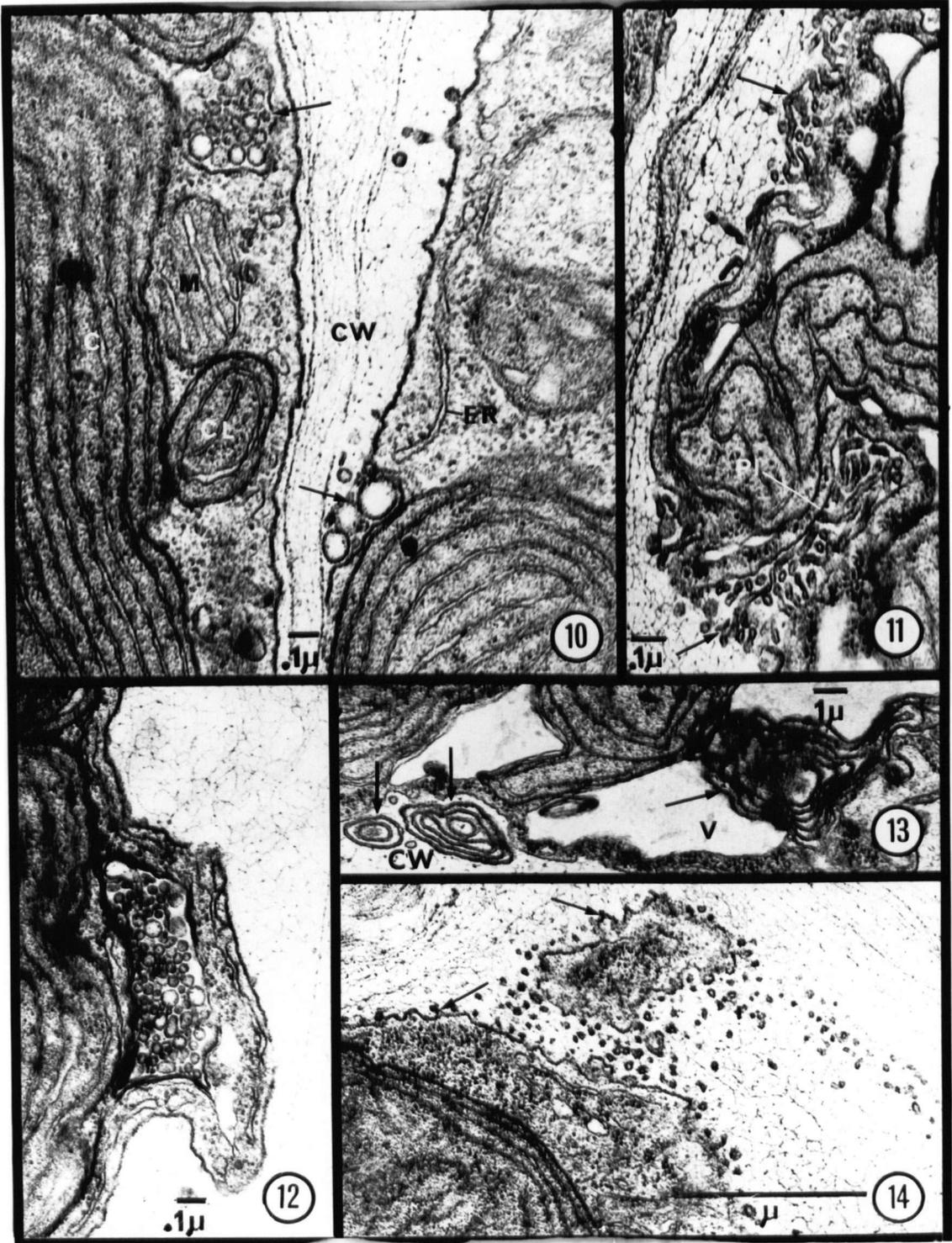
Fig. 12. A larger multivesicular body consisting mainly of spherical vesicles.

Fig. 13. Lamellar bodies (arrows) situated in the cell wall and near vacuoles.

Fig. 14. Numerous single vesicles in the cell wall near the plasmalemma.

Arrows indicate vesicles still attached to the plasmalemma.

X



b) Erythrotrichia¹

Observations.

The species used in this study were E. carnea (Pl. XI, Fig. 1), E. boryana (Pl. XIII, Fig. 6) and E. pulvinata (Pl. XV, Fig. 11). At first observation the ultrastructural similarity of these plants to Smithora naiadum (Section IVa) is very evident. Indeed, the cell wall of E. boryana (Pl. XIII, Fig. 7) and E. pulvinata (Pl. XV, Fig. 12) appears identical to that of Smithora, consisting of progressively more compacted structural fibrils within an electron transparent matrix. However, the wall of E. carnea differs somewhat in containing electron transparent areas in the outermost layers as well as a loosely fibrillar material on the surface of the filament (Pl. XII, Fig. 5). E. pulvinata exhibits an interesting modification in possessing a darkly staining line in the cell wall adjacent to the plasmalemma (Pl. XVI, Fig. 16). This line is particularly noticeable in regions where the plasmalemma is most convoluted and could represent an area of highly compressed structural fibrils.

The protoplast of E. carnea (Pl. XI, Fig. 2) is much more regular in outline than that of E. boryana (Pl. XIII, Fig. 7) and E. pulvinata (Pl. XV, Fig. 12). The actual shape of the protoplast varies considerably, presumably according to the rate of cell division (West, 1966).

All species of Erythrotrichia examined feature a central, stellate chloroplast (Pl. XI, Fig. 2; Pl. XIII, Fig. 7; Pl. XV, Fig. 12). Sections through chloroplast material show that the majority of thylakoids are situated parallel to and follow the contour of the chloroplast envelope (Pl. XI, Fig. 2; Pl. XII, Fig. 3; Pl. XIII, Fig. 7; Pl. XV, Fig. 12).

¹Parts of this section will appear in an article by D.L. McBride and K. Cole in the Proceedings of the VIIth International Seaweed Symposium. Sapporo 1972.

E. carnea was the only plant to regularly exhibit phycobilisomes on chloroplast lamellae (Pl. XII, Fig.3). They are similar to those described in Porphyridium (Gantt and Conti, 1965,1966; Gantt, Edwards and Conti, 1968). Ribosome-like bodies were often observed between chloroplast lamellae (Pl. XIV, Fig. 10). In addition, possible DNA-containing areas (Bisalputra and Bisalputra, 1967) and droplet-like inclusions are scattered between the lamellae.

A constant feature of the chloroplast is the presence of a centrally located pyrenoid traversed by varying numbers of lamellae (Pl. XII, Fig. 3; Pl. XIII, Fig. 7; Pl. XVI, Fig. 14) which often contain a fibrillar material (Pl. XII, Fig.4). In E. carnea the number of traversing lamellae seen in one sectional plane was seldom greater than five and usually these structures ran in a relatively straight line through the pyrenoid (Pl. XII, Fig. 3). However, in E. boryana and E. pulvinata there are large numbers of highly convoluted lamellae within the pyrenoid matrix (Pl. XIII, Fig. 7; Pl. XV, Fig. 12; Pl. XVI, Fig. 14).

The nucleus is typically eukaryotic (Pl. XI, Fig. 2; Pl. XII, Fig. 3; Pl. XIV, Fig. 9; Pl. XV, Fig. 12) and only very rarely is the outer membrane of the nuclear envelope continuous with cisternae of ER (Pl. XIV, Fig. 9). This feature is common in many other plants (Ledbetter and Porter, 1970).

Remaining cell organelles and inclusions such as mitochondria (Pl. XVI, Fig. 16), dictyosomes (Pl. XVI, Fig. 16), vacuoles (Pl. XI, Fig. 2; Pl. XIII, Fig. 7; Pl. XV, Fig. 12) and floridean starch grains (Pl. XIV, Fig. 9) resemble those described in the vegetative cell of Smithora (Section IVa).

An intriguing structural characteristic of algal epiphytes, especially those which exhibit a host specificity, is the zone of attachment. E. pulvinata thus far appears to be exclusively epiphytic on the utricles of Codium fragile (Pl. XV, Fig. 11) but this zone shows no evidence of interalgal

protoplasmic association (Pl. XVI, Fig. 13). The respective cell walls are merely joined in a simple, smooth plane. In this region, foreign objects are occasionally embedded in the cell wall of the epiphyte. One such object bore a resemblance to a bacterial cell (Pl. XVI, Fig. 15). E. boryana is less host specific than E. pulvinata. It is of interest that E. boryana is epiphytic on Smithora since these algae are almost ultrastructurally identical. In sections through the zone of attachment it is nearly impossible to differentiate the respective cell walls (Pl. XIV, Fig. 8).

Discussion.

An ultrastructural comparison of closely related algal species has proven to be of taxonomic value in certain instances. In particular, "less advanced" red algae which have few definite morphological characteristics to utilize may lend themselves to this procedure. Evan's (1970) study of Rhodella maculata has shown the usefulness of this approach.

The three species of Erythrotrichia chosen for this study appear to illustrate a maximum morphological variability within the genus, but very few concrete ultrastructural differences were noted. The most constant difference observed was the form and smaller number of pyrenoid traversing lamellae in the chloroplast of E. carnea as compared with E. boryana and E. pulvinata. Cole (1971) has reported such a variability among the conchocelis phases of three species of Porphyra. Hori (1971) has noted differences in the ultrastructure of the thylakoid system within the pyrenoid among species of Monostroma (Chlorophyceae). Dodge and Crawford (1971) also noted a different pyrenoid structure among certain species of Dinoflagellata. In addition, the ultrastructural appearance of the cell wall in E. carnea seems to separate this species from the others. West (1966) has also discussed certain unusual features of the wall of E. carnea.

One must be extremely cautious in the interpretation of interspecific ultrastructural differences as they could merely be due to variations in habitat, season, age of the plant, stage of the life cycle, etc. Indeed, Hori (1972) has shown ultrastructural differences which occur between different stages in the life cycle of the same species of Monostroma.

The presence of phycobilisomes in E. carnea is interesting in view of the general absence of these structures in the other members of the Erythropeltidaceae examined. Gantt and Conti (1965) state that these structures are very sensitive to fixation conditions. However, all material used in this study was fixed in the same manner. In fact, in E. carnea it was possible to observe phycobilisomes in one cell but not in an adjacent cell of the same filament. If their presence depends on the quality of chemical fixation, one can only speculate why cells in the same filament of approximately the same age should react differently to these procedures.

Thus, E. carnea, E. boryana and E. pulvinata represent distinct species on a gross morphological level but show few ultrastructural differences. In addition, the fine structure of the genus Erythrotrichia appears to be similar to that of Smithora suggesting that these genera are closely related members of the Erythropeltidaceae. It could also offer further support of Hollenberg's (1959) taxonomic reassignment of the latter genus.

PLATE XI.

Erythrotrichia carnea

Fig. 1. Light micrograph of portion of filament.

Fig. 2. Longitudinal section through filament illustrating cell wall (W), chloroplast (C) with centrally located pyrenoid (P), nucleus (N) and vacuoles (V).

XI

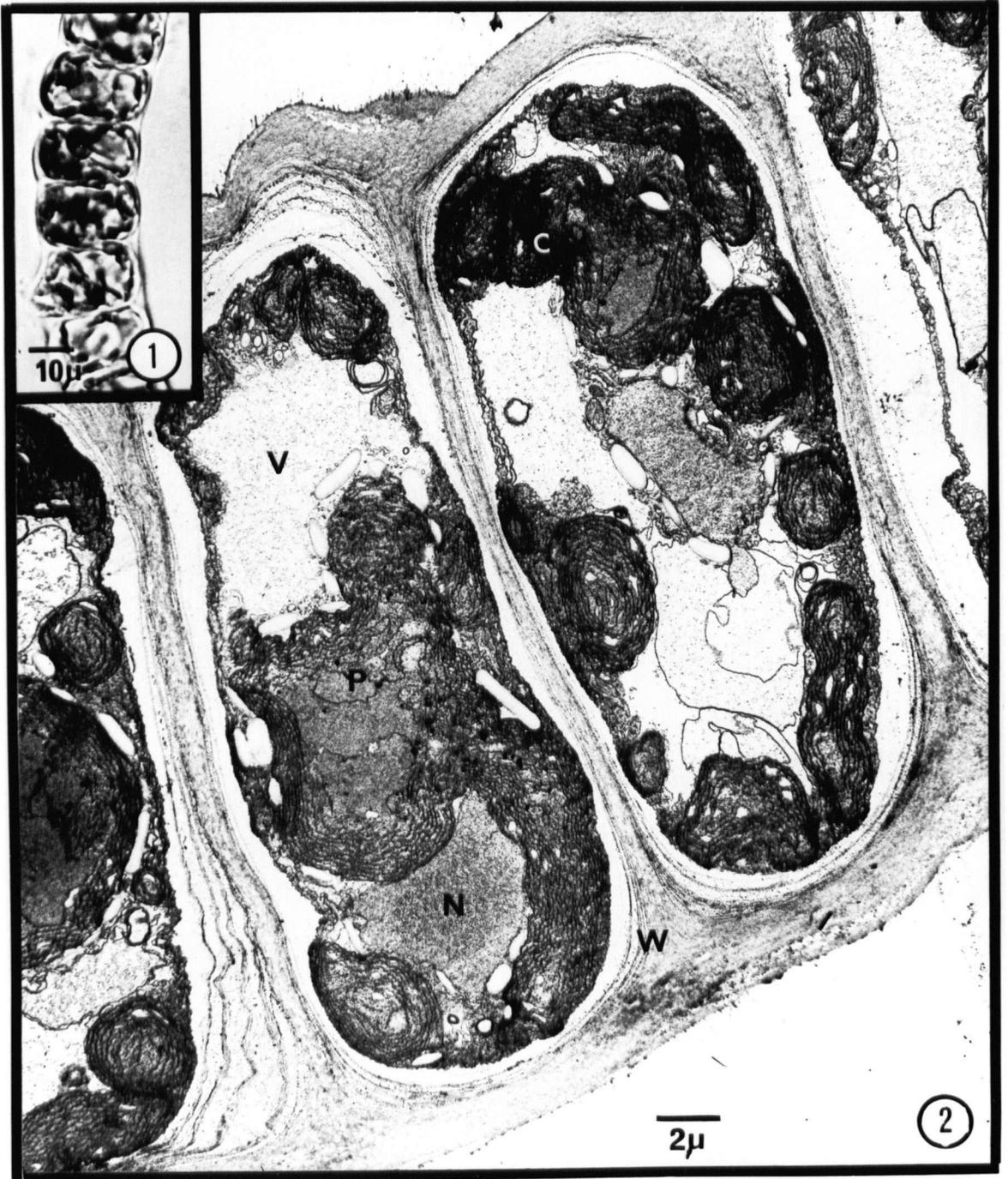


PLATE XII.

Erythrotrichia carnea

Fig. 3. Portion of cell showing nature of lamellae which traverse the pyrenoid (P) in the chloroplast (C). Note the rough texture of the thylakoids indicating the presence of phycobilisomes. Prominent nucleus (N) and cell wall (W) are also shown.

Fig. 4. Portion of pyrenoid with swollen lamellae containing loosely organized fibrils.

Fig. 5. Cell wall containing electron transparent areas (T) and fibrillar material (arrow) on outer surface.

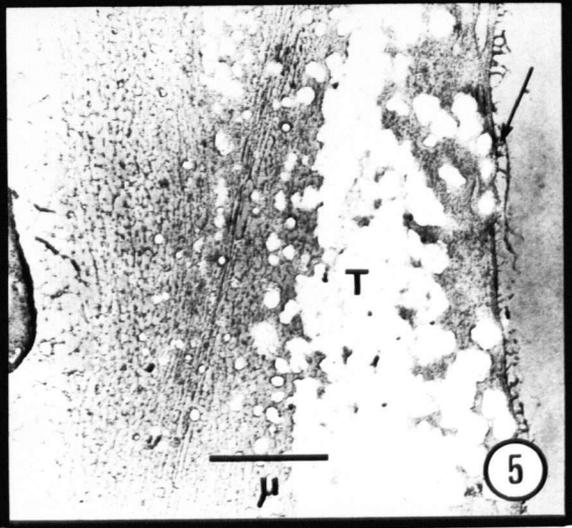
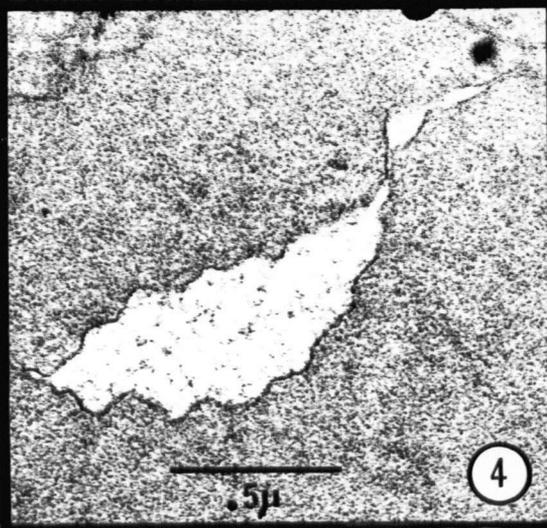
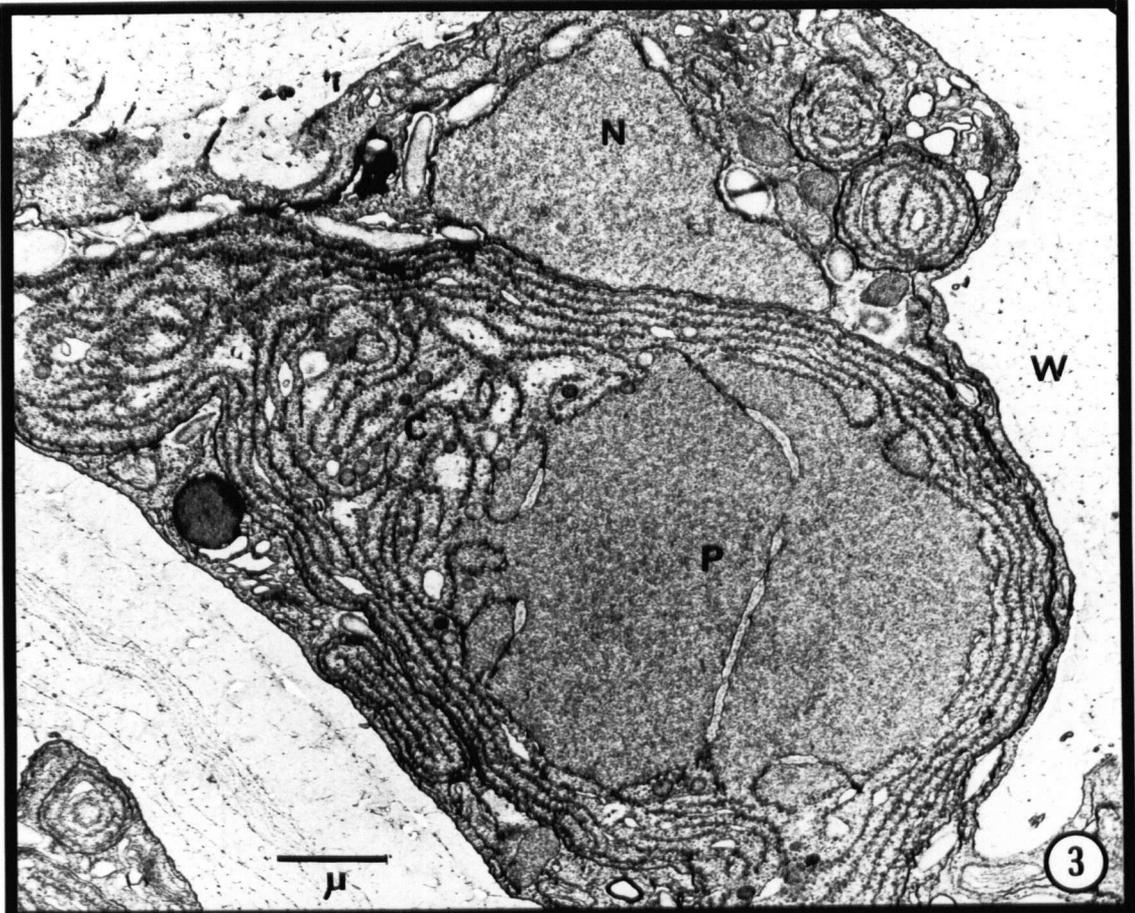


PLATE XIII.

Erythrotrichia boryana

Fig. 6. Light micrograph of portion of filament.

Fig. 7. Longitudinal section through filament showing cell wall (W), chloroplast (C) with pyrenoid (P) and vacuoles (V).

XIII

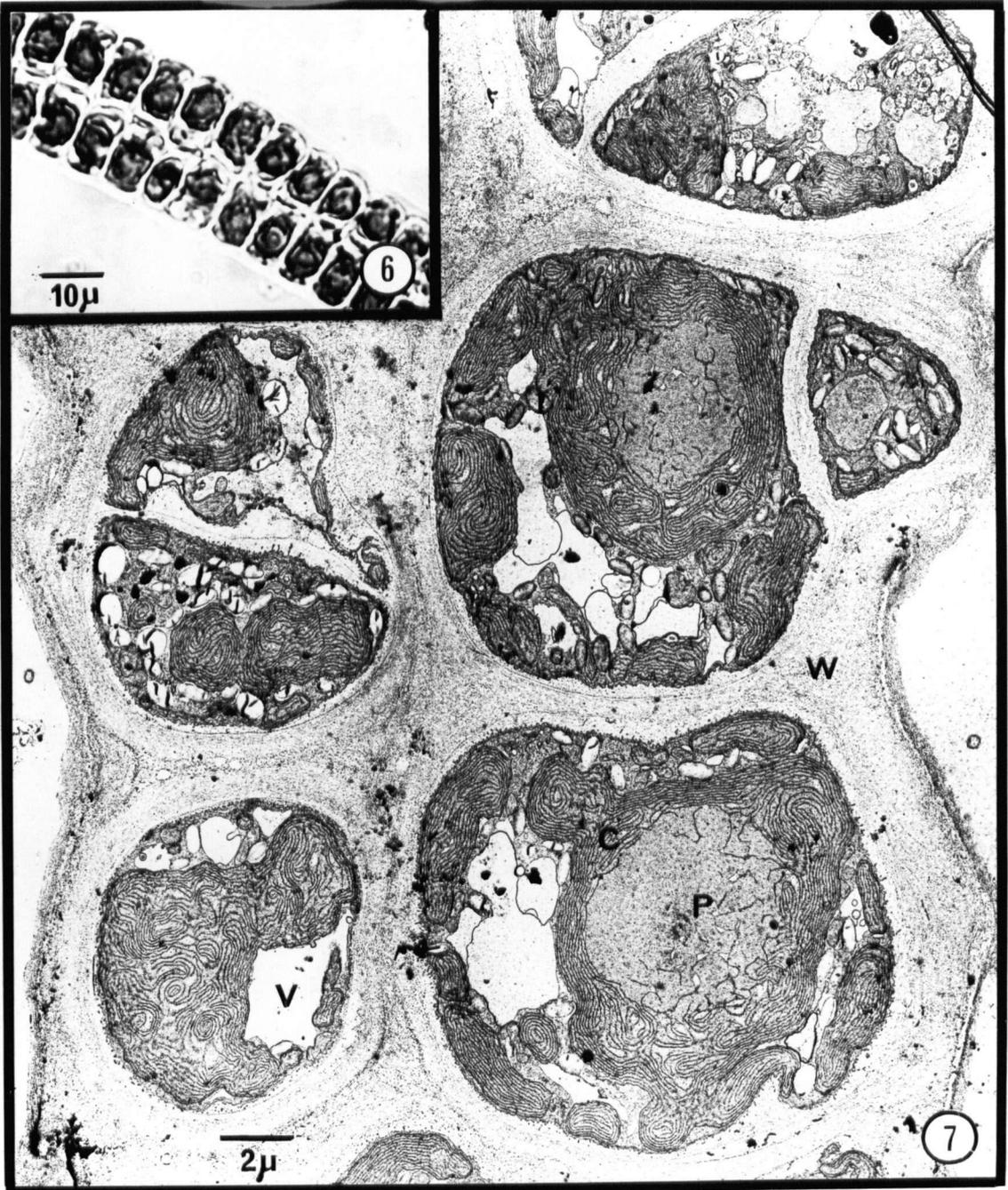


PLATE XIV.

Erythrotrichia boryana

Fig. 8. Section through the zone of attachment between E. boryana and Smithora. EP denotes Erythrotrichia protoplast and SP denotes Smithora protoplast.

Fig. 9. Portion of nucleus (N) illustrating ER-nuclear envelope connection (arrow). FS indicates floridean starch grain.

Fig. 10. Portion of chloroplast lobe showing electron dense ribosome-like bodies (arrow).

XIV

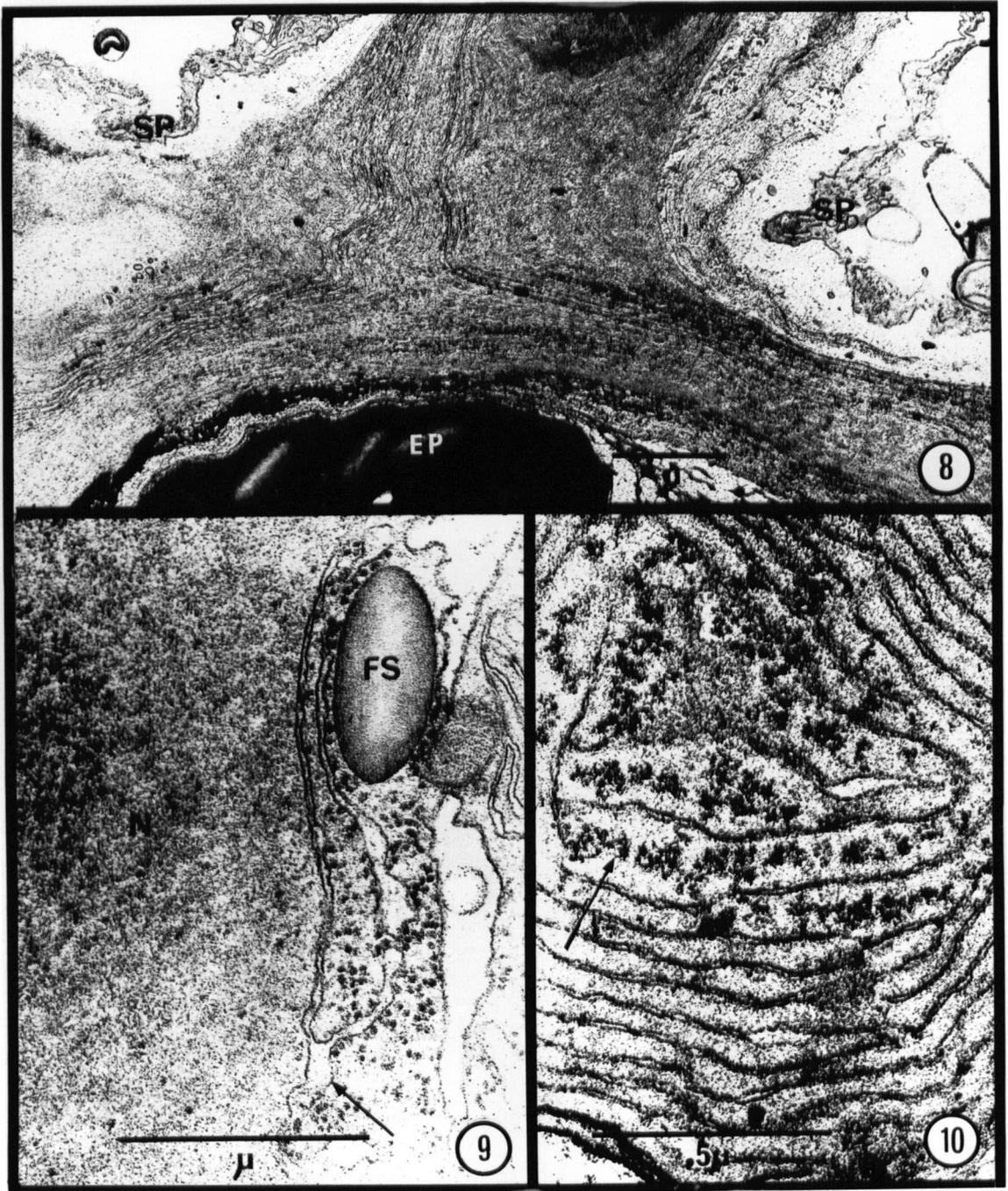


PLATE XV.

Erythrotrichia pulvinata

Fig. 11. Light micrograph of basal cushion of plant attached to utricle of Codium fragile.

Fig. 12. Section through basal part of plant with chloroplast (C), pyrenoid (P), nucleus (N), vacuoles (V) and cell wall.

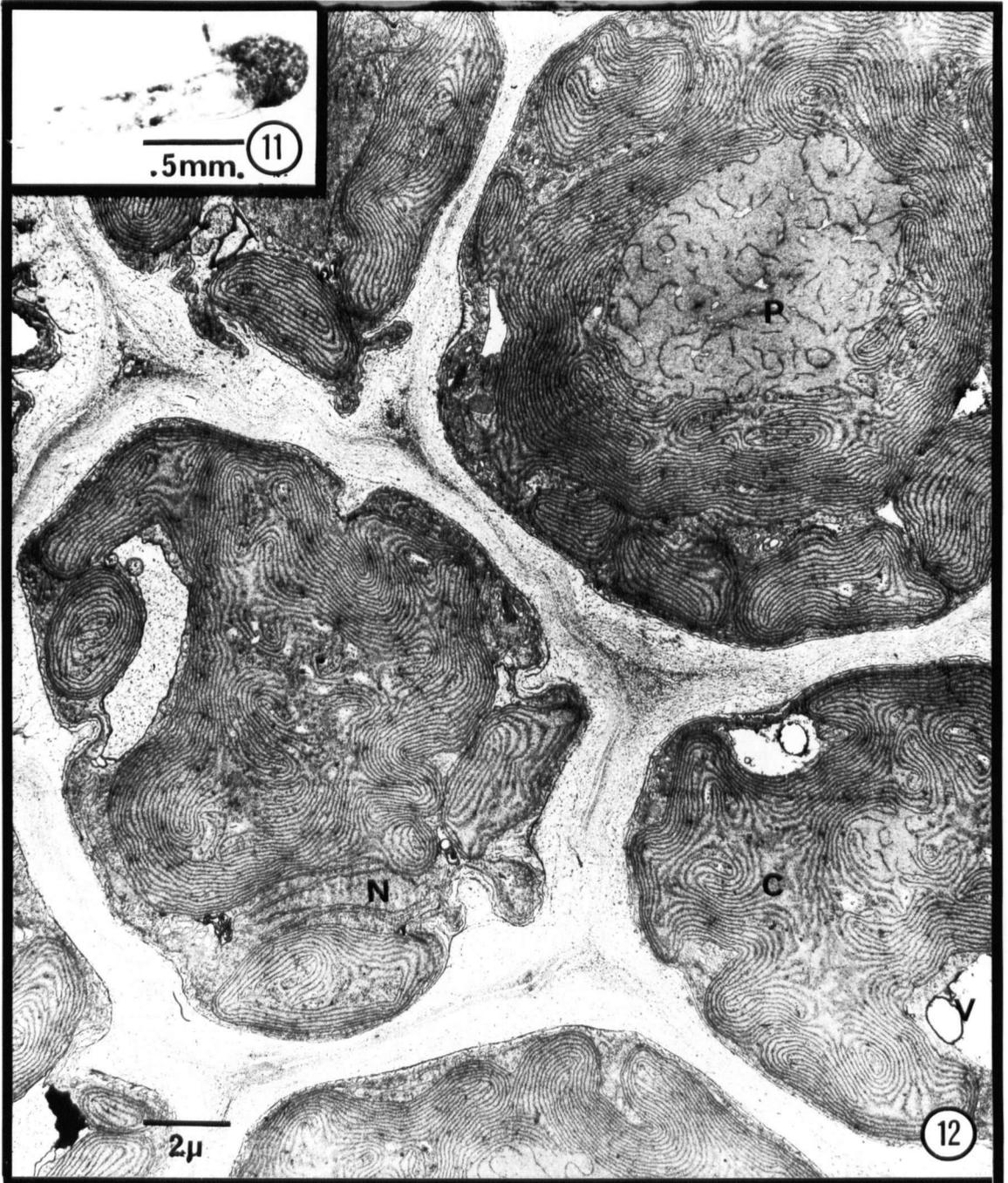


PLATE XVI.

Erythrotrichia pulvinata

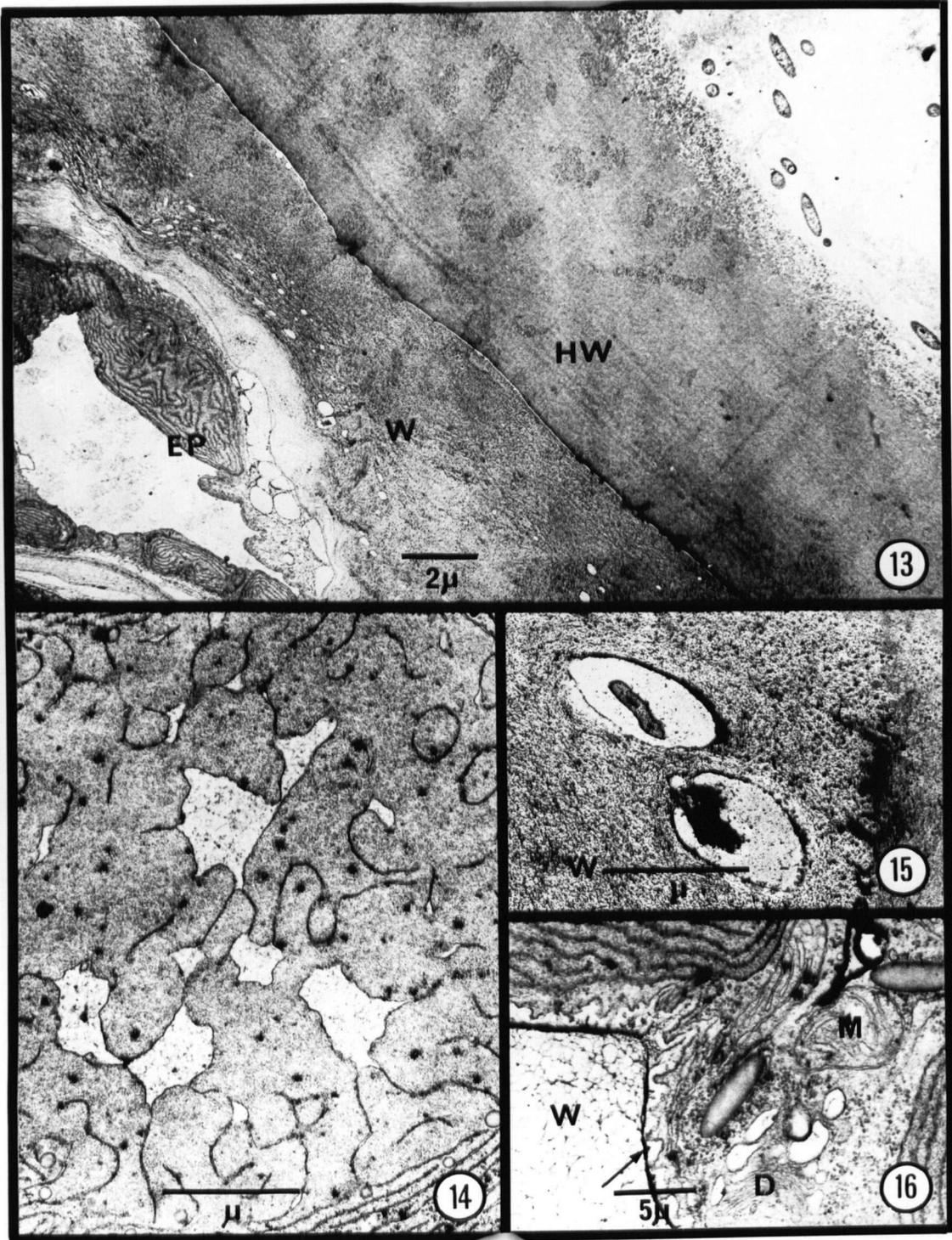
Fig. 13. Electron micrograph illustrating attachment zone of alga. Cell wall of host alga (Codium fragile) (HW), wall of E. pulvinata (W) and protoplast of E. pulvinata (EP) are shown.

Fig. 14. Section through pyrenoid showing convoluted traversing lamellae. Note the loosely organized fibrils within the lamellae.

Fig. 15. Area of attachment with foreign objects embedded in wall of E. pulvinata (W).

Fig. 16. Peripheral region of protoplast with mitochondria (M), a dictyosome (D) and cell wall (W). Arrow indicates darkly staining material in wall adjacent to plasmalemma.

XVI



V. ULTRASTRUCTURAL ASPECTS OF MONOSPOROGENESIS

a) Monospore differentiation, release and degeneration.¹

Introduction.

The differentiation of whole vegetative cells into spores is a common means of asexual reproduction in the Bangiophycidae (see Drew, 1956 for review). The term "monospore" has been applied to reproductive cells produced in this manner, although there is still some difficulty in formulating acceptable terms to distinguish between the different spore-like cells evident in this group.

During the present study, Smithora and Erythrotrichia were observed in culture as well as in field conditions and it is evident that monospore production constitutes one of the main methods of species propagation. A discussion of light microscopic work on the production of these structures in the Erythropeltidaceae is presented in Section III of this report.

A majority of the ultrastructural investigations carried out on algal spore production have dealt with various aspects of chlorophycean zoosporogenesis, e.g. Stigeoclonium (Manton, 1964), Oedogonium (Hoffman, 1968; Pickett-Heaps, 1971), Bulbochaete (Retallack and Butler, 1970), Tetracystis (Brown and Arnott, 1970) and Enteromorpha (Evans and Christie, 1970). In the red algae, Peyriere (1969) has described certain features of the tetrasporangium of Griffithsia and Tripodi (1971) has reported some fine structural characteristics of the cystocarp of Polysiphonia. To my knowledge there are no published electron microscopic accounts of monosporogenesis in the Bangiophycidae.

¹This portion of the thesis is partially based on a publication by D.L. McBride and K. Cole in Phycologia 10, 49-61 (1971). The text of the original article has been brought up to date by including recent references where appropriate.

Ultrastructural observations on the aging and degeneration of algal cells have been sparse. Schuster, Hershenov and Aaronson (1968) have described aspects of this phenomenon in Ochromonas (Chrysophyceae) and Palisano and Walne (1972) in Euglena (Euglenophyceae).

Observations.

Monospore differentiation: The monosporogenous area of Smithora naiadum is sharply delimited from the vegetative thallus and is usually observed as a band of more deeply pigmented, rounder cells (Pl. XVII, Fig. 1). At a light microscope level, the cells in this area appear to be undergoing mitotic division at a greater rate than the adjacent vegetative cells. This, in conjunction with an apparent reduction in vacuolar area, results in the differentiating cells appearing smaller than the vegetative cells in surface view, although the actual thickness of the thallus is greater in the sporulating areas. At an ultrastructural level, the most obvious manifestations of the transition to the monospore are the loss of the very irregular protoplast outline exhibited in the vegetative cell and the reduction in vacuolar area (Pl. XVII, Fig. 2). As the spore matures, the shape of the protoplast progresses from regularly oblong to almost spherical and increases in size from 10-20 microns to 20-30 microns in surface view.

Like that of the vegetative cell, the nucleus of the developing monospore exhibits a typical eukaryotic structure (Pl. XVIII, Fig. 3). Perhaps the most conspicuous difference is the presence of a larger number of pores in the nuclear envelope of the developing spore. When viewed in tangential section the pores are circular and in many cases arranged in a linear order. (Pl. XVIII, Fig. 7), like those described in Bumilleria (Xanthophyceae) (Massalski and Leedale, 1969). Also, as reported by the above authors, the nuclear pores appear to be more concentrated in certain areas of the nuclear

envelope (Pl. XVIII, Fig. 6). Structurally they are similar to those described in pea seedlings, consisting of several central granules and a surrounding annulus composed of numerous subannuli (Yoo and Bayley, 1967). Intimate associations between the chloroplast and the nuclear envelope were frequently observed (Pl. XVIII, Fig. 4). At these regions a dense precipitate of stain often occurred in the prepared material.

Ribosome-laden endoplasmic reticulum is invariably observed adjacent to the nuclear envelope of the developing spore (Pl. XVIII, Fig. 5). Often the cisternae may number 12 or more. Only a few cisternae were noted in this position in the vegetative cell. It is of interest that the distance between the nuclear envelope and the ER is greater at the areas of the nuclear envelope which exhibit a large number of pores (Pl. XVIII, Fig. 6). Usually a few cisternae of predominantly smooth ER are associated with the plasmalemma (Pl. XIX, Fig. 8). In addition, increased quantities of smooth and rough ER were observed in other areas of the differentiating monospore (Pl. XVII, Fig. 2; Pl. XVIII, Fig. 3).

The mitochondria are typical in appearance; possessing a double envelope, tubular cristae and electron dense inclusions (Pl. XVIII, Fig. 5). However, there appears to be an increase in the number of these organelles in the developing spore. Mitochondria often appear in the vicinity of dictyosomes, but no strict relationship such as that described in Corallina (Bailey and Bisalputra, 1970) and Griffithsia (Peyrière, 1969) was observed in this material.

The chloroplast of the developing monospore is irregularly lobed and exhibits single lamellae which are largely orientated parallel to the chloroplast envelope (Pl. XVII, Fig. 2). It is very similar to that of the vegetative cell except that the interlamellar spaces appear to enlarge

somewhat and acquire a granular appearance.

Many floridean starch grains are present in the differentiating spores and are generally larger than those observed in the vegetative cell (Pl. XVIII, Fig. 3). Thin sectioning usually resulted in folding of the plastic in areas of starch granules. This can be seen as an electron dense line through these structures (Pl. XVII, Fig. 2). Evidently Evans (1970) experienced the same problem with Rhodella (Porphyridiales).

The dictyosome appears to be very active in the differentiating spore. Throughout development, the cell contains a large number of these organelles in varying degrees of hypertrophy (Pl. XVII, Fig. 2). The swollen cisternal stacks give rise to two distinctly different products. The first is formed early in the development of the spore. Large dictyosomes with their maturing faces toward the centre of the cell produce irregular vesicles containing a compacted fibrillar substance (Pl. XIX, Fig. 8). Occasionally the forming faces of these organelles are associated with ER and small vesicles which appear to emanate from it. The large fibrillar vesicles originating from the dictyosomes coalesce shortly after formation, resulting in large, membrane bound deposits within the cell (Pl. XVII, Fig. 2; Pl. XIX, Fig. 11,12). Rough ER is frequently associated with these deposits (Pl. XIX, Fig. 10). Occasionally cellular components such as floridean starch granules appear to become incorporated within the vacuole-like structures (Pl. XIX, Fig. 9).

Toward the latter stages of the accumulation of the fibrillar product, a second substance begins to form. Hypertrophied dictyosomes, located randomly in the cell, form smaller, more spherical vesicles (Pl. XX, Fig. 14). These vesicles contain loosely organized fibrils in an electron transparent matrix. In the stages immediately prior to and after spore

release the cell is filled with these structures. Concomitant with the deposition of this material is the marked enlargement and rounding of the cell. In these latter stages of development both the densely fibrillar vacuoles and the smaller vesicles are present in the cell (Pl. XIX, Fig. 11).

Contrary to Hollenberg's (1959) light microscopic observation, no intercellular connections were observed between developing spores. In contrast to the vegetative cell, it is of interest that very few lomasome-like bodies were observed. Concentric lamellar structures were also few in number. This is in keeping with results obtained in two other red algae: Porphyridium (Gantt and Conti, 1965) and Batrachospermum (Brown and Weier, 1970), which indicate that these structures are present to a greater extent in older, less active cells.

Prior to spore release the thickness of the cell wall is considerably less than in the vegetative thallus. Wall production has evidently not kept pace with the enlargement of the monospore. The area immediately adjacent to the plasmalemma is more compacted, possibly due to the pressure of the enlarged spore or to the accumulation of released fibrillar material (see below) (Pl. XX, Fig. 15).

The ultrastructural details of monospore differentiation in Erythrotrichia (Pl. XXV, Fig. 31) are identical to those in Smithora: the dictyosome forms two products, each of which is accumulated in the cytoplasm prior to spore release (Pl. XXVI, Fig. 32-34).

Monospore release: Immediately preceding and during liberation of the monospore, the vacuole-like structures migrate toward the periphery of the cell (Pl. XIX, Fig. 12) and subsequently expel their fibrillar contents (Pl. XIX, Fig. 13). The mechanism of release involves a fusion of the vacuole

membrane to the plasmalemma. Occasionally a few of the smaller vesicles are released as well, but the majority remain behind.

The extrusion of a single spore is achieved by a breakage in the cell wall on one or the other side of the thallus (Pl. XX, Fig. 16,17). Since the development of the cells in one particular sorus seems to be more or less synchronous, the sorus is often released as a whole (Pl. XX, Fig. 19; Pl. XXII, Fig. 22). This could be the result of a greater stress on the cell walls in the proximal area of the sorus. Such mass release appears to occur more often than the release of single spores. However, unless the entire sorus settles immediately following release it is most probable that further wall dissolution takes place resulting in single spores being liberated.

There is evidence that a small amount of cytoplasm remains in the thallus after spore release (Pl. XX, Fig. 17,18). This material invariably appears degenerate. Perhaps this pocket of cytoplasm which contains a nucleus (Pl. XX, Fig. 18), is the result of an asymmetric division of the vegetative protoplast prior to differentiation. It appears to be similar to the pale, protoplasmic remnant occurring in this manner and remaining after spore release in Membranella nitens (Hollenberg and Abbott, 1968).

The released monospore is spherical and approximates 15-25 microns in diameter (Pl. XXI, Fig. 20,21). The spore is bounded by a single plasmalemma, the exterior of which may be associated with a quantity of mucilage (Pl. XXII, Fig. 22; Pl. XXIV, Fig. 29). No vestige of cell wall material from the parent thallus remains. This concurs with reports of naked monospores in other red algae by various authors including Sommerfeld and Nichols (1970). The monospore is filled with the smaller, loosely fibrillar vesicles produced prior to release. Many of these vesicles coalesce after spore

liberation to form larger, vacuole-like structures (Pl. XXI, Fig. 21; Pl. XXIII, Fig. 23).

The chloroplast seems to be somewhat modified in the monospore. Closely appressed lamellae often occur within the lobes giving the appearance of "pseudogranum-like" structures (Pl. XXIII, Fig. 23,24). As many as 20 lamellae may be involved in these formations. Although these structures could be associated with an altered plastid metabolism, it is perhaps more likely that they are due merely to the physical effect of pressure resulting from the accumulation of products in the spore. In addition, thylakoid associations like those reported in the vegetative cell were observed (Pl. XXIII, Fig. 24). The pyrenoid is characterized by a number of swollen traversing lamellae which often appear closely appressed and frequently contain some osmiophilic droplets (Pl. XXIII, Fig. 25).

The notable lack of unoccupied cytoplasmic matrix in the monospore severely restricts the distribution of other organelles. Mitochondria are often closely packed and confined to a small area (Pl. XXIV, Fig. 27). The nucleus is usually surrounded by a layer of cytoplasm containing a small amount of ER (Pl. XXIV, Fig. 28).

Some dictyosome activity is noted in the mature monospore (Pl. XXIV, Fig. 26). These organelles appear to be adding to the already abundant vesicular material. Numerous floridean starch granules remain in the spore and seem to be isolated from the remaining bits of cytoplasm.

Due to a lack of cell wall material, the differentiated monospore appears to be very delicate. In many instances the pressure of the cellular contents in combination with outside stimulus causes the plasmalemma to rupture, resulting in a flow of vesicular contents out of the cell (Pl. XXI, Fig. 21). In addition, the plasmalemma is frequently observed to bleb out

quantities of this substance (Pl. XXIV, Fig. 30).

Monospore degeneration: Although monospores will germinate readily under culture conditions, a certain percentage of these structures undergo degeneration within several days after release. At a light microscopic level, the chief proclamation of this phenomenon is the gradual loss of the characteristic red pigmentation of the spore. Ultrastructurally there is an ordered breakdown of each organelle system. Initially, ER and dictyosomes become less active and inconspicuous. Chloroplast lamellae appear to swell and become disorganized (Pl. XXVII, Fig. 1). Abnormally bloated mitochondria, which often show indications of inner membrane disruption, are typical of the degenerating spore (Pl. XXVII, Fig. 1; Pl. XXVIII, Fig. 2). Paramural bodies (Marchant and Robards, 1968) are increasingly evident near the periphery of the cell (Pl. XXVII, Fig. 1) and extensive, progressive vacuolation of the cytoplasm occurs (Pl. XXVII, Fig. 1; Pl. XXVIII, Fig. 2,3).

Disruption of the nuclear envelope and the subsequent breakdown of this organelle may signal the onset of cell death (Pl. XXVIII, Fig. 3). In later stages, the chloroplast material is almost completely disorganized. Lamellae are grossly distended and membrane destruction is evident (Pl. XXVIII, Fig. 4). Finally the plasmalemma breaks, possibly as the result of uncontrolled osmotic pressures, and the spore rapidly disintegrates. Numerous uncoordinated membrane fragments are characteristic of the cellular debris (Pl. XXVIII, Fig. 5).

Discussion.

In the field, the chief manifestation of the subcellular changes involved in the production of monospores is a deeper pigmentation in the differentiating areas. The electron microscope has allowed a more detailed observation of the process. Not only is there an increase in dictyosome

activity but also a substantial increase in the amount of ER and the number of floridean starch granules and mitochondria. Such changes would suggest that the production of monospores in the Erythropeltidaceae examined is not merely a release of rounded vegetative cells, but involves a complex, active metamorphosis. However, an exact explanation of all the cellular activities must be based on extended physiological and cytochemical studies.

It is evident that the formation of monospores depends greatly on the dictyosome. This organelle is implicated in the extensive production of two distinct products during sporogenesis which may play an important role in the release and attachment of the spore. The first product, a compacted fibrillar substance, is aggregated in large vacuole-like structures. Since substantial amounts of it are expelled immediately prior to and during spore release, it is hypothesized that this substance functions as a mucilaginous secretion. Knox (1926) noted that a large amount of mucilaginous matter is associated with monospore release in Smithora. Indeed, it has been observed in the current study that the mucilage is produced in copious amounts and could aid in spore release by acting as a lubricant. This could be critical to the success of the process as liberation takes place through a small opening in the cell wall. In addition, the mucilage would protect the fragile spore after release. The vacuolar structures containing it bear a resemblance to the electron opaque spheres described by Bouck (1962) in the gland cells of Lomentaria baileyana. Bouck suggested an association between the gland cells and mucilage production. The production of such a substance was also noted in Porphyridium and Pseudocloioiphloea by Ramus (1972), who described it as a polyanionic polysaccharide of high molecular weight.

The second product associated with dictyosome activity is contained within smaller, more electron transparent vesicles, the greater percentage

of which are formed after production of the larger, more densely fibrillar structures. Since this compound is secreted primarily after spore liberation, is produced in large quantities and is released very easily by rupturing the fragile, unprotected plasmalemma, it is hypothesized that it acts as a cement-like substance essential to the success of the reproductive process. The ability of the monospores of Smithora to adhere to various objects was mentioned by Knox (1926). In fact, not only do these spores stick to the host plant securely, but also adhere to one another. So effective is this biological cement, that it is very difficult to remove the settled spores from plastic containers. Since Smithora and Erythrotrichia are epiphytic, the advantage of such a substance would be tremendous. The cement would facilitate a rapid, secure attachment of the spores to the host plant before tide and wave action could remove them from this location. Again, the timing of the secretion would be relatively critical. Too early a release could cause the spores to adhere to the parent thallus thus restricting dispersal. Manton (1964) and Evans and Christie (1970) have proposed a similar function for the large accumulation of vesicular structures in the zoospores of the green algae Stigeoclonium and Enteromorpha respectively.

In recent studies on zoosporogenesis in Oedogonium (Chlorophyceae), Pickett-Heaps (1971) has also described two distinct populations of dictyosomes, each concerned with the production of a different substance. In addition, Tripodi (1971), working with cystocarpic material of Polysiphonia, has noted two product accumulations which appear to be structurally similar to those in Smithora and Erythrotrichia. He suggests that the role of these structures may be involved with a reserve function, although he does not relate any ultrastructural events leading to carpospore release.

The large amount of rough ER in the developing spore suggests an

increase in metabolic rate, particularly in regard to protein synthesis. In a classical sense, the larger number of nuclear pores could facilitate transport of messenger RNA to the nearby rough ER for efficient translation into manufactured products. In addition, the precipitate found at areas of the nuclear envelope in close proximity to the chloroplast envelope could be the result of a transfer or buildup of a particular compound or compounds. Associations between the nuclear envelope and the chloroplast envelope surrounding the pyrenoid have been reported in Rhodella (Evans, 1970) and Asteromonas (Prasinophyceae) (Peterfi and Manton, 1968). Nuclear-chloroplast associations have been shown in other algae (reviewed in Cole and Lin, 1968) and more recently in Symbiodinium (Dinophyceae) (Taylor, 1969) where a continuity between the chloroplast matrix and the nucleoplasm was found.

The cell wall of the parent thallus must undergo extensive changes to allow the monospores to escape. The thickness of the cell wall is definitely reduced but there must also be some loss of structural stability. Perhaps the cell walls in the vicinity of the monospore undergo an actual decomposition or degradation to permit the release of whole sori or individual spores. Whether this change is the result of an enzymatic digestion, physical internal pressure or external environmental effects remains open to speculation.

The shape of the released spore is spherical indicating a certain amount of pressure is exerted on the plasmalemma by the contents of the spore. However, some degree of flexibility is important to the structure in order to facilitate an easier release from the thallus. Plate XX (Fig. 16) illustrates the stress which must be exerted on the spore as it squeezes through the narrow passage in the wall.

In contrast to the differentiating monospore, the spore on release seems very quiescent. Organelles are crowded into small spaces between

copious amounts of vesicular product. Presumably this would restrict circulation of metabolic precursors and the organelles themselves, resulting in decreased synthetic activity prior to attachment of the mature monospore.

The consistency of the ultrastructural characteristics of monosporogenesis in Smithora and Erythrotrichia indicate that such subcellular activities are probably carried out by all members of the Erythropeltidaceae. Indeed, studies of other spore producing algae (Evans and Christie, 1970; Pickett-Heaps, 1971; Tripodi, 1971) have indicated that certain facets of this process may be common.

The most critical period of monospore reproduction appears to be the time between release from the parent thallus and initiation of cell wall construction. Released algal spores are subjected to numerous environmental hazards which must be overcome to ensure the success of the reproductive process. As exemplified by the erythropeltidacean monospore, these structures are often fragile and easily destroyed by outside influences. A biological mechanism which is thought to compensate for this involves the production of larger numbers of spores, presumably on the premise that the greater the number released, the more that will survive. It would be extremely difficult to estimate the percentage of surviving spores and undoubtedly such a hypothetical proportion would vary considerably under different environmental conditions. It is quite possible that in this study conditions of laboratory culture were responsible for a certain percentage of degeneration.

There is little known of the ultrastructural aspects of degeneration in algae, although a considerable amount of research has been carried out with the phenomena of senescence and death in higher plants. The mitochondrial component appears to be particularly susceptible to ultrastructural changes

in the degenerating monospore. Similar mitochondrial swelling and disruption of the inner membrane have been noted in senescing wheat leaves (Shaw and Manocha, 1965), chemically treated oat roots (Hanchey, Wheeler and Luke, 1968) and senescing oat leaves (De Vecchi, 1971). Nuclear breakdown (Shaw and Manocha, 1965) and cytoplasmic vesiculation (Ragetli, Weintraub and Lo, 1970) in senescing leaf cells are also similar to that reported in the present study. In addition, my observations tend to agree with Hanchey, Wheeler and Luke's (1968) suggestion that large numbers of paramural bodies may be indicative of cells destined to undergo disintegration.

Unfortunately, when studying degeneration in conditions approaching those of the natural environment, it is difficult to speculate on the causes of this phenomenon. Certain studies have been carried out on senescing plant material under different controlled conditions (e.g. De Vecchi., 1971), but it is obvious that further research on such artificial systems is needed to permit the formulation of definite conclusions on the mechanisms of plant degeneration.

PLATE XVII.

Smithora. Differentiating monospore

Fig. 1. Light microscopic surface view of proximal portion of sorus (s) and adjacent vegetative area (vg).

Fig. 2. Section parallel to surface of thallus illustrating differentiating cell with chloroplast lobes (c), dictyosomes (d), endoplasmic reticulum (er), mitochondria (m), floridean starch grains (fs), fibrous vacuole-like structures (fvl) and surrounding cell wall (w).

XVII



PLATE XVIII.

Smithora. Differentiating monospore

Fig. 3. Portion of nucleus (n) with nucleolus (nu) and surrounding organelles.

Arrow denotes DNA-containing area in chloroplast.

Fig. 4. Area of intimate association between limiting envelopes of nucleus (n) and chloroplast (c). Arrow denotes precipitate of stain.

Fig. 5. Portion of nucleus (n) with associated cisternal stack of rough ER. A dictyosome (d) and a mitochondrion (m) are also labelled.

Fig. 6. Portion of nucleus (n) illustrating porous nature of envelope (arrows) in certain restricted areas.

Fig. 7. Tangential section through nuclear envelope illustrating linear sequence of pores and annular structure.

XVIII

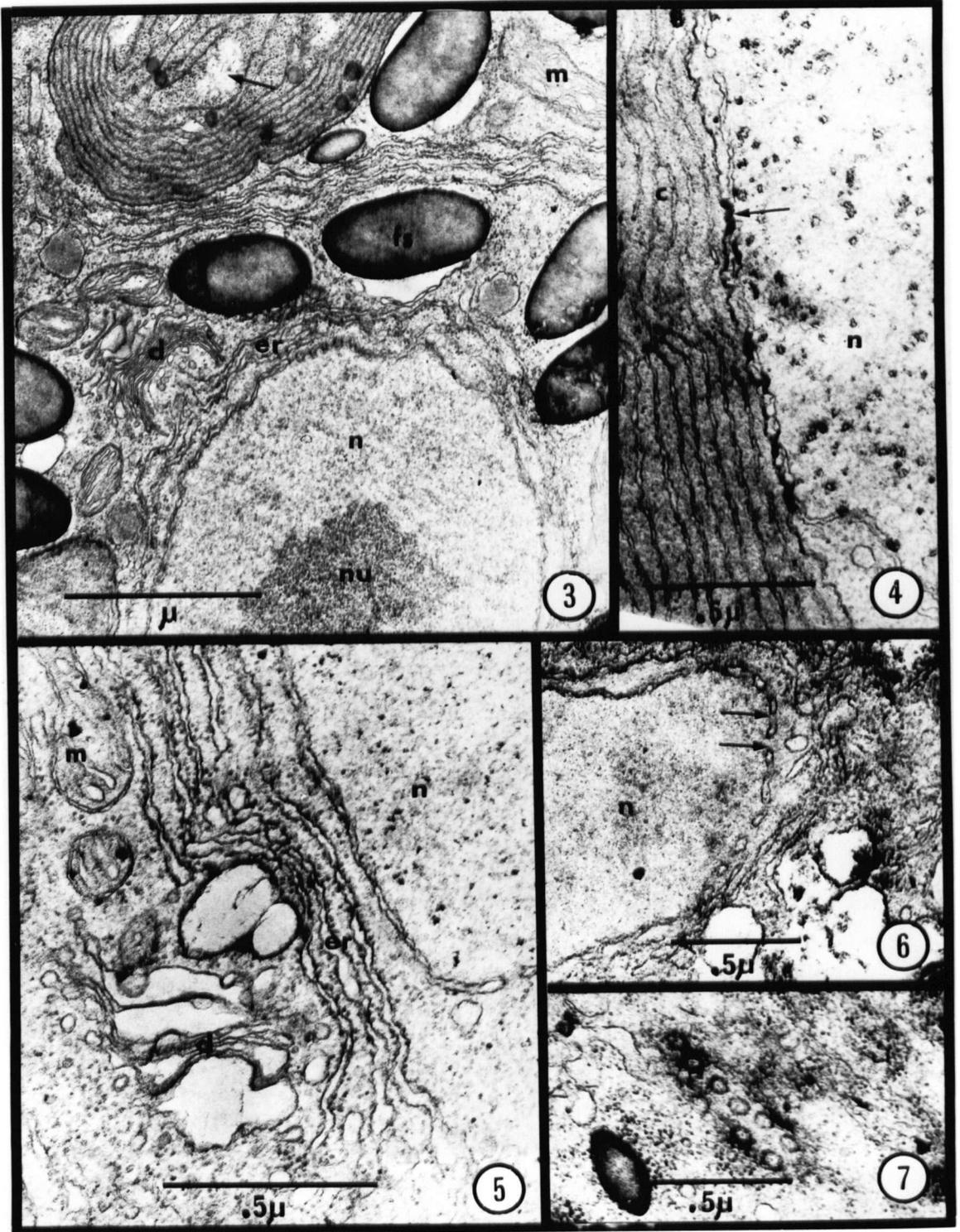


PLATE XIX.

Smithora. Differentiating monospore

Fig. 8. Dictyosome (d) with large irregular fibrous vesicles (fv) forming from maturing face. Note vesicles (arrow) and ER associated with forming face near cell wall.

Fig. 9. Floridean starch grain (fs) included within fibrous vacuole-like structure (fvl).

Fig. 10. Association between ER and fibrous vacuole-like structure (fvl). Cell wall (w) and a dictyosome (d) are also labelled.

Fig. 11. Smaller, more electron transparent vesicles (v) and large fibrillar vacuole-like structures (fvl) concurrently present in the developing spore.

Fig. 12. Large, vacuole-like structures (fvl) appressed to periphery of protoplast near cell wall (w).

Fig. 13. The vacuole-like structures (fvl) releasing contents through ruptured plasmalemma during liberation of spore. Note presence of smaller vesicles (v) and mitochondria (m).

XIX

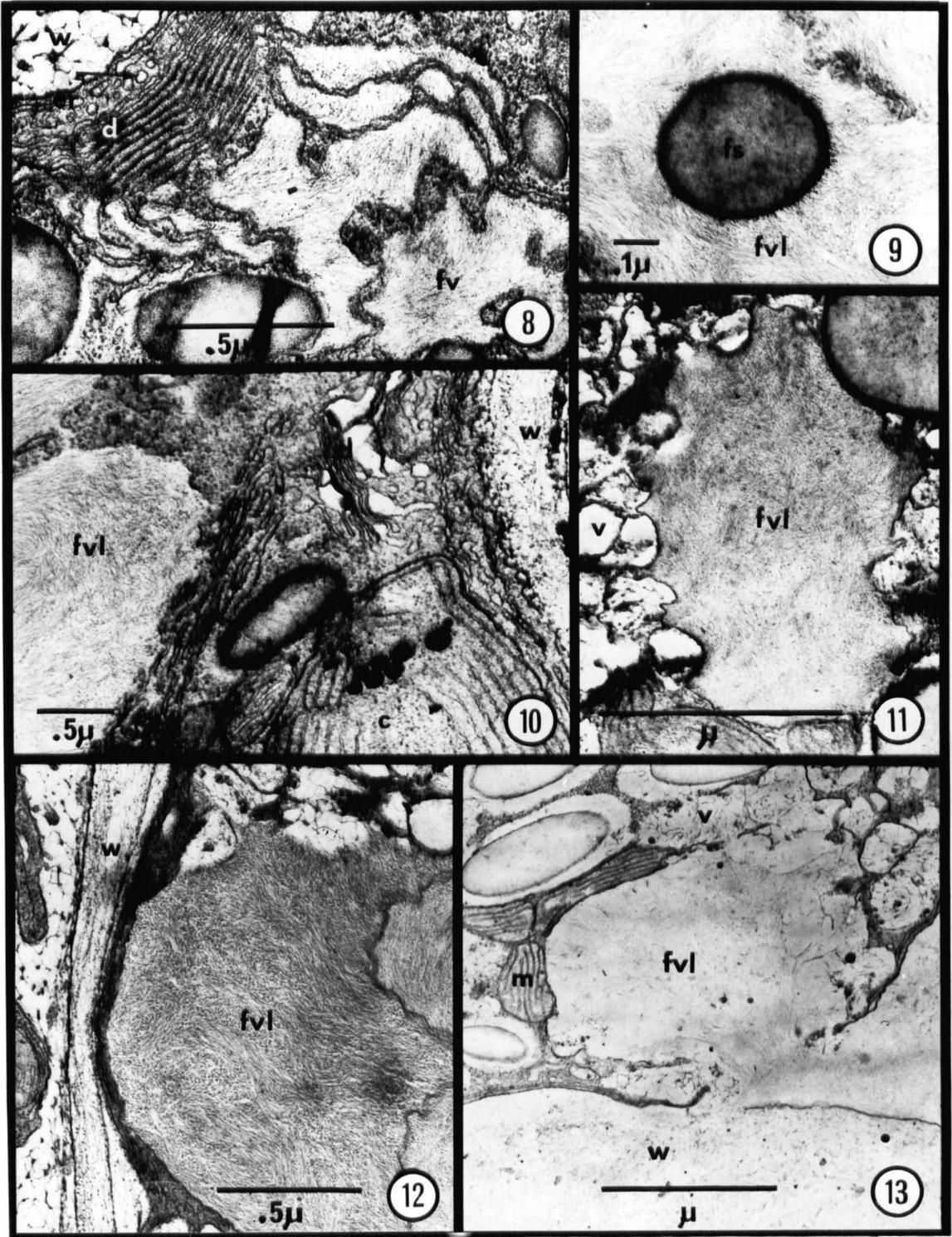


PLATE XX

Smithora. Differentiating spore

Fig. 14. Dictyosomes (d) producing smaller, more electron transparent vesicles (v) near cell wall (w).

Fig. 15. Compacted area of cell wall (w) immediately adjacent to plasmalemma (arrow) prior to spore release.

Fig. 16. Cross-section of thallus showing monospore being liberated. Note presence of both types of deposits (v, fvl) and distortion of spore during release. ex indicates area external to thallus.

Fig. 17. Cross-section of thallus after spore liberation. Single arrow denotes remaining cytoplasm. Double arrow denotes break in cell wall (w) through which a monospore was released.

Fig. 18. Cytoplasm remaining in thallus after spore release. Cell wall (w), nucleus (n), mitochondria (m) and chloroplast material (c) are labelled. ex denotes area external to thallus.

Fig. 19. Photomicrograph of spores being liberated en mass (ms) and intact sorus (s).

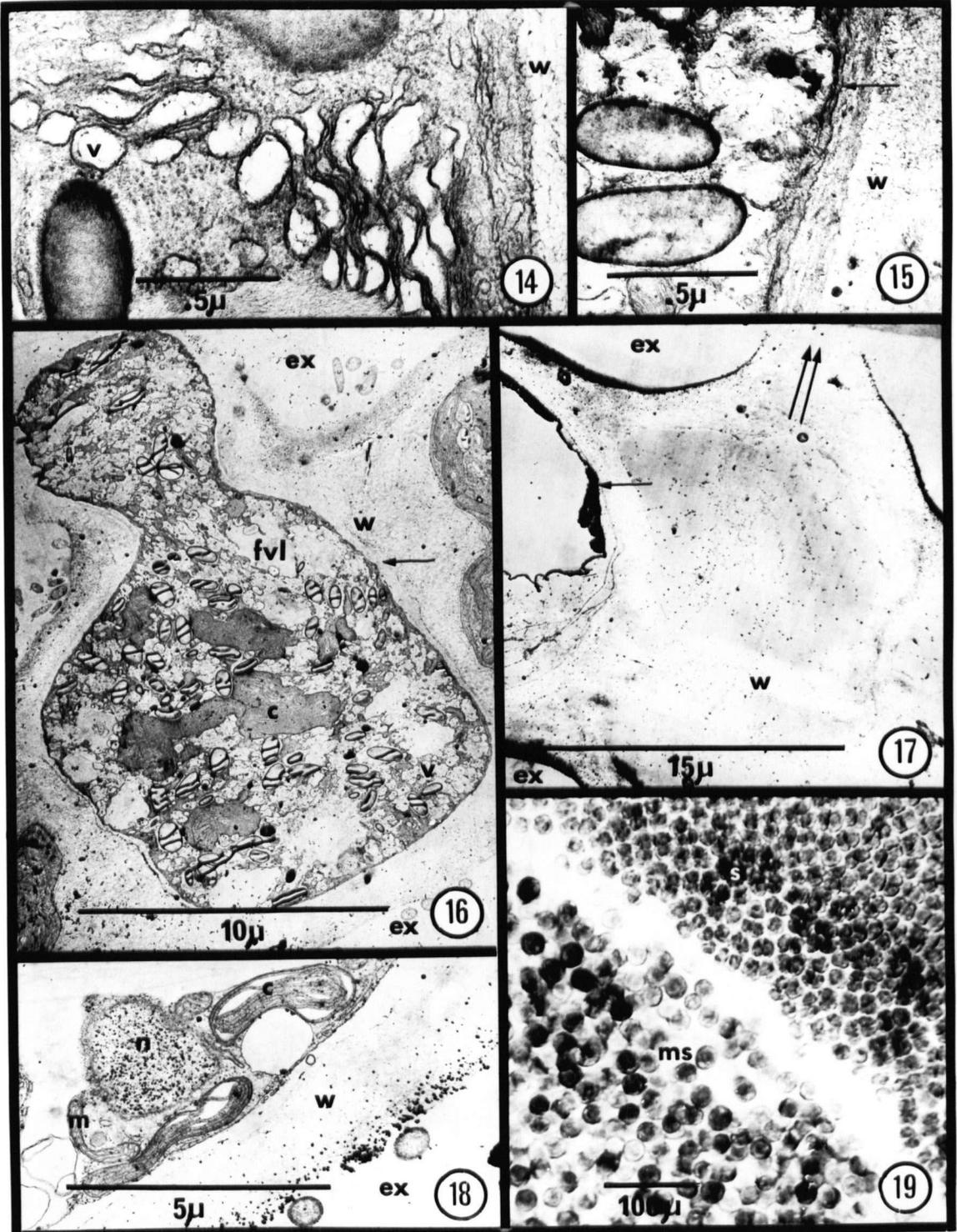


PLATE XXI

Smithora. Released monospore

Fig. 20. Photomicrograph of liberated monospore.

Fig. 21. Electron micrograph of liberated monospore. Note central, lobed chloroplast (c) with pyrenoid (p) and coalesced electron transparent vesicles (cv). Single arrow denotes unprotected plasmalemma and double arrow denotes release of contents of electron transparent coalesced vesicles. A nucleus (n) is also labelled.

XXI



PLATE XXII

Smithora. Released monospore

Fig. 22. Section through released deciduous sorus consisting of closely adhering monospores.

XXII

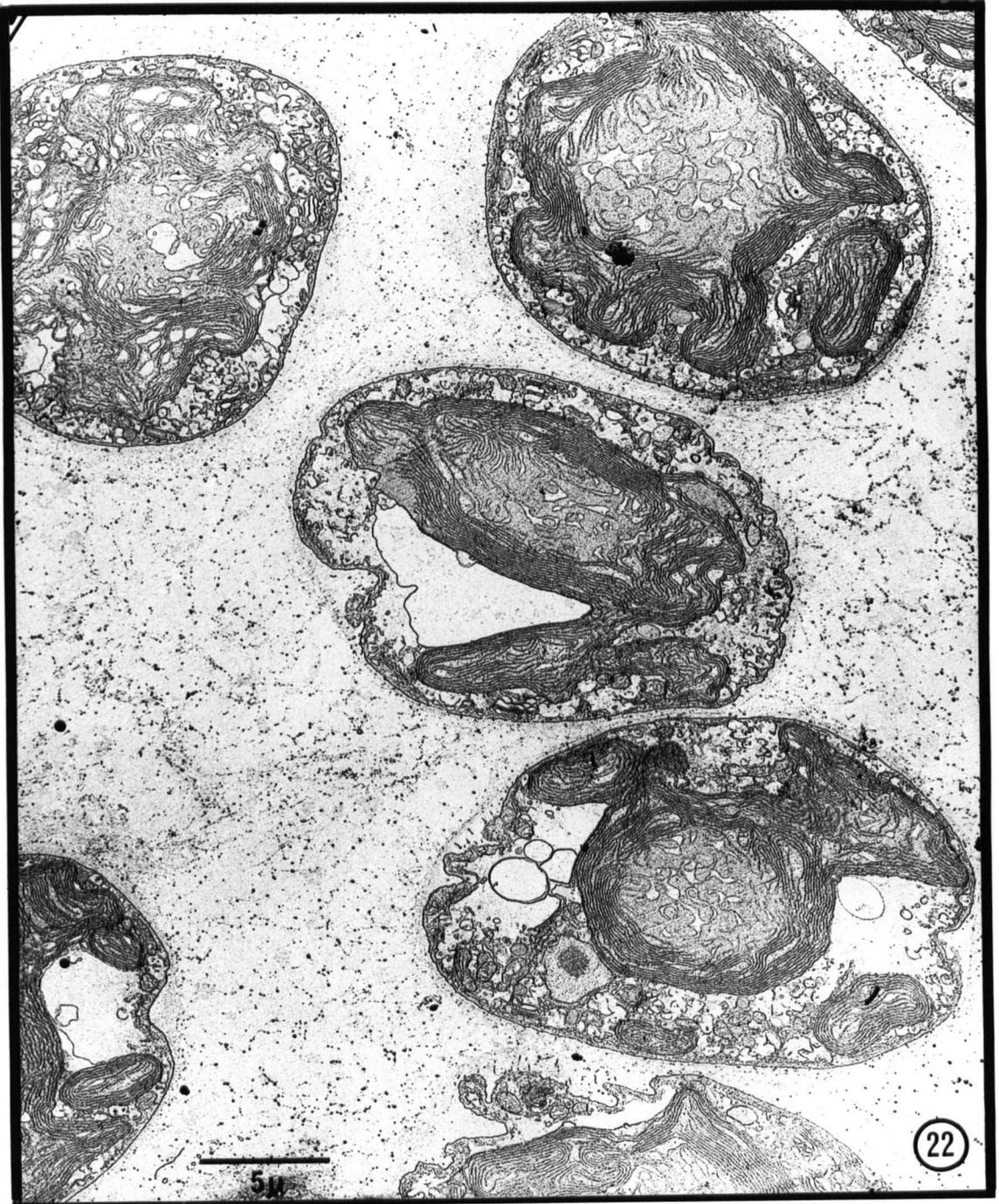


PLATE XXIII

Smithora. Released monospore

Fig. 23. Lobe of chloroplast with centrally located appressed lamellae and coalesced electron transparent vesicles (cv).

Fig. 24. Lobe of chloroplast with appressed lamellae (double arrow) and thylakoid association (single arrow).

Fig. 25. Portion of pyrenoid showing osmiophilic droplets (od) within swollen traversing lamellae.

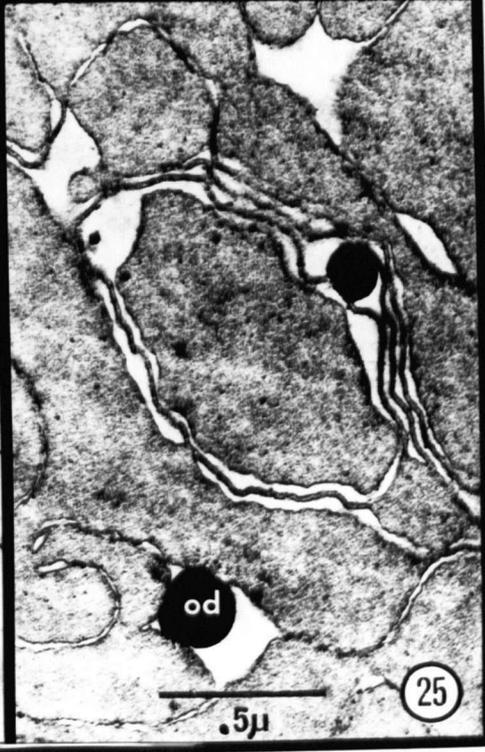
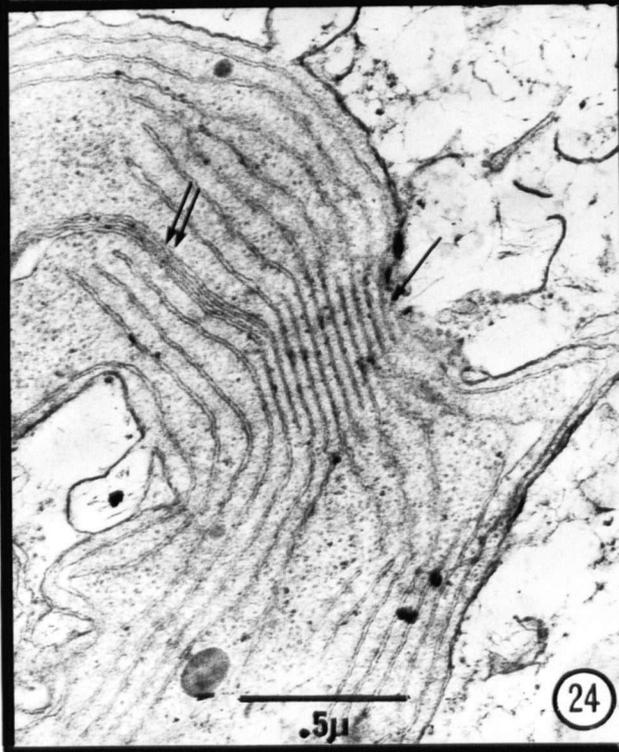
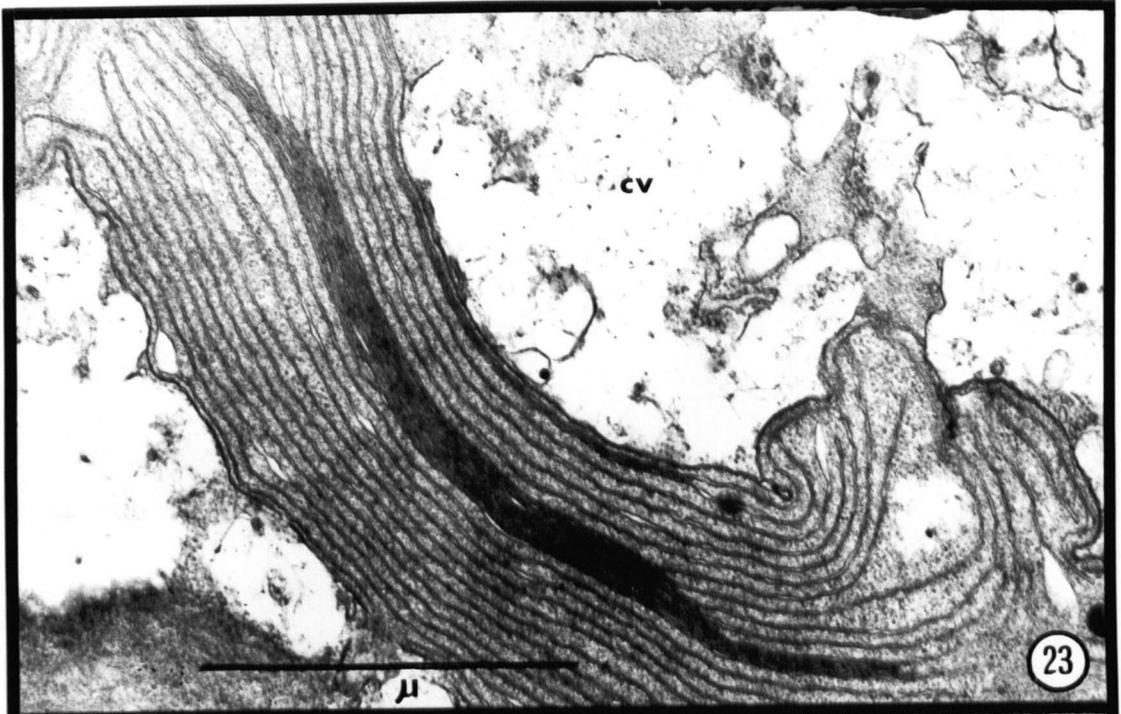


PLATE XXIV

Smithora. Released monospore

Fig. 26. Dictyosome activity in mature monospore (production of electron transparent vesicles (v)).

Fig. 27. Four closely packed mitochondria. A floridean starch granule and coalesced vesicles (cv) are also shown.

Fig. 28. Nucleus (n) and nucleolus (nu) of mature monospore. Note small amount of ER (arrow) next to the nuclear envelope.

Fig. 29. Single, unprotected plasmalemma of mature spore. ex denotes area external to spore which may include mucilaginous material.

Fig. 30. Release of vesicular contents (v) by a blabbing of the plasmalemma. ex denotes area external to spore.

XXIV

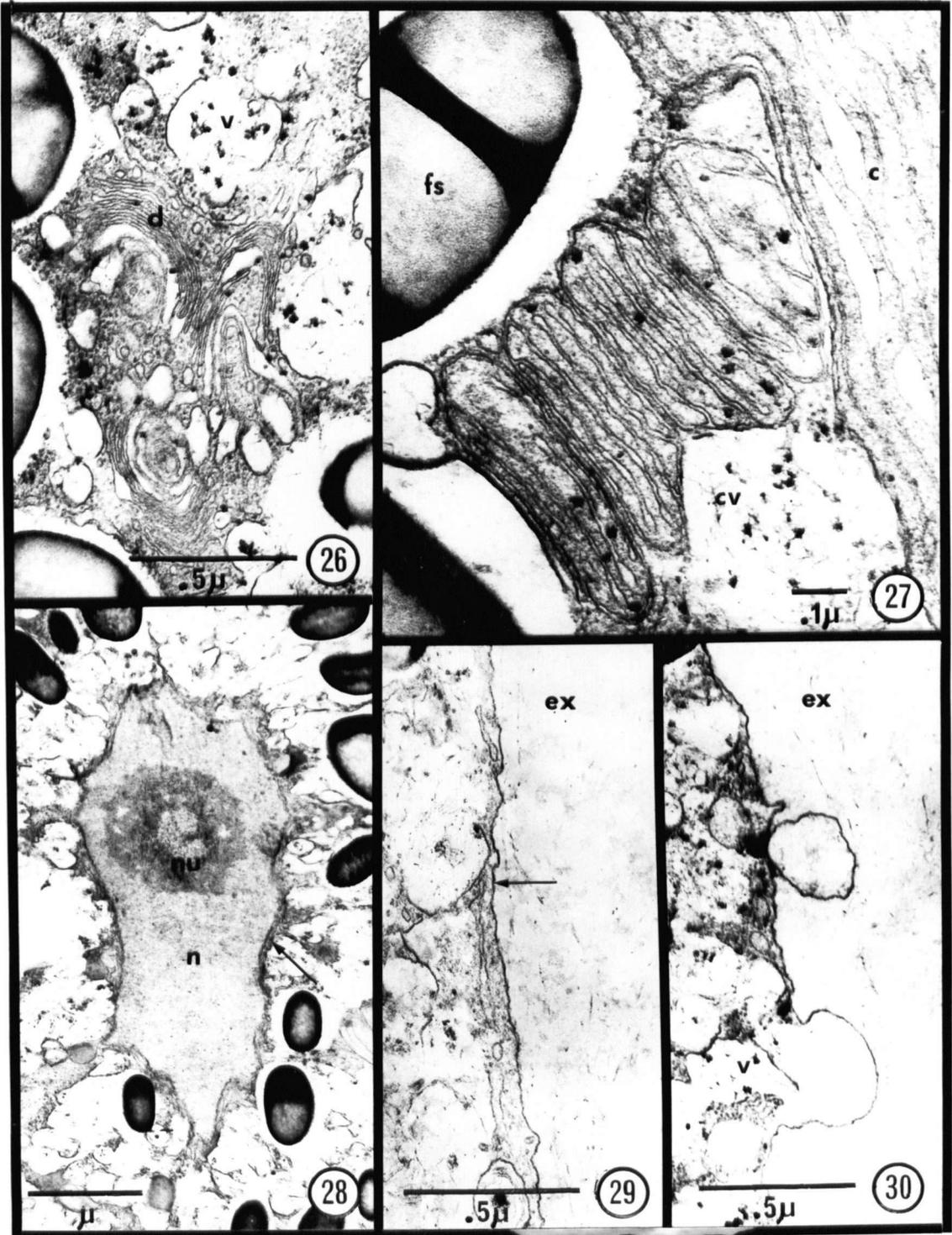


PLATE XXV

Erythrotrichia boryana

Fig. 31. Unreleased monospore within cell wall (w) showing chloroplast (c) with pyrenoid (p), nucleus (n) and floridean starch grains (fs).

XXV



PLATE XXVI

Erythrotrichia

Fig. 32. E. boryana. Portion of differentiating monospore within cell wall (w) illustrating nature of the two products (cv, fvl) originating from the dictyosome (single arrow). Prominent nucleus (n) surrounded by ER is also shown.

Fig. 33. E. carnea. Portion of differentiating monospore. Note fibrillar vacuole-like structures (fvl) and dictyosome (arrow).

Fig. 34. E. pulvinata. Description as for Fig. 33.

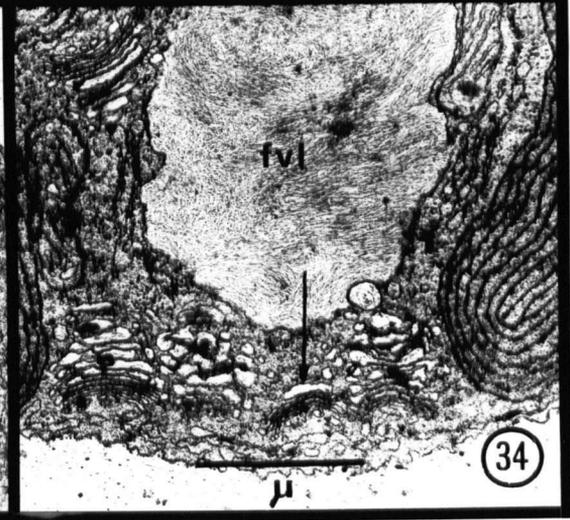
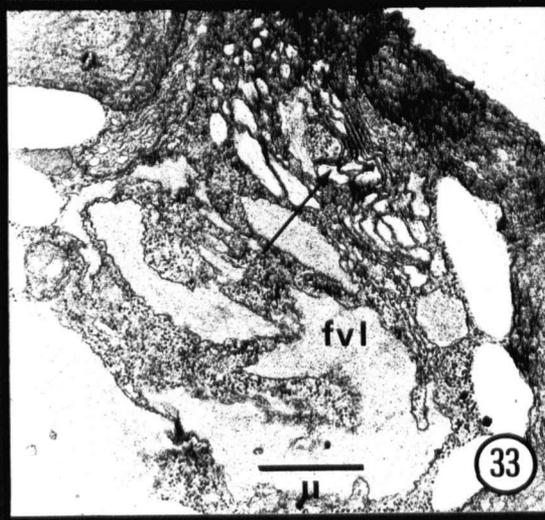
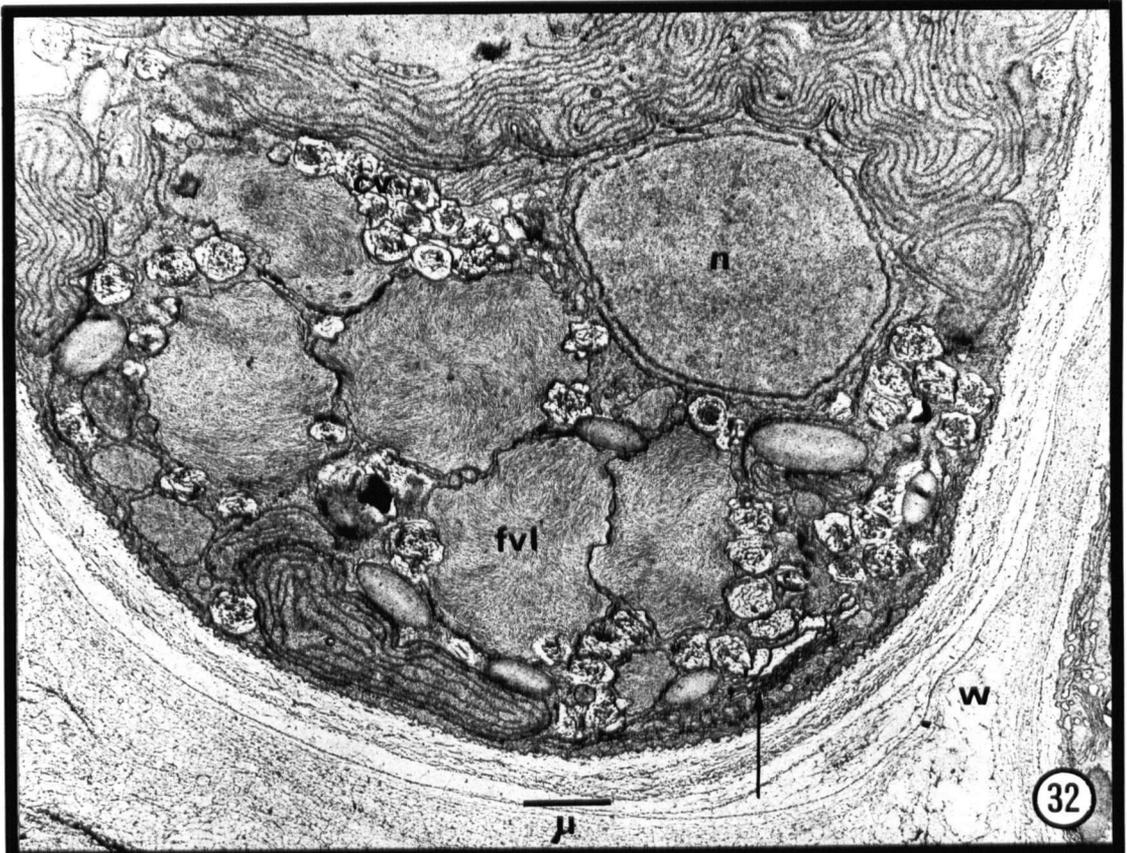


PLATE XXVII

Smithora. Degenerating monospore

Fig. 1. Monospore in early stages of degeneration exhibiting swollen chloroplast lamellae (arrow), swollen mitochondria (m), floridean starch (fs) and paramural bodies (double arrow).

XXVII

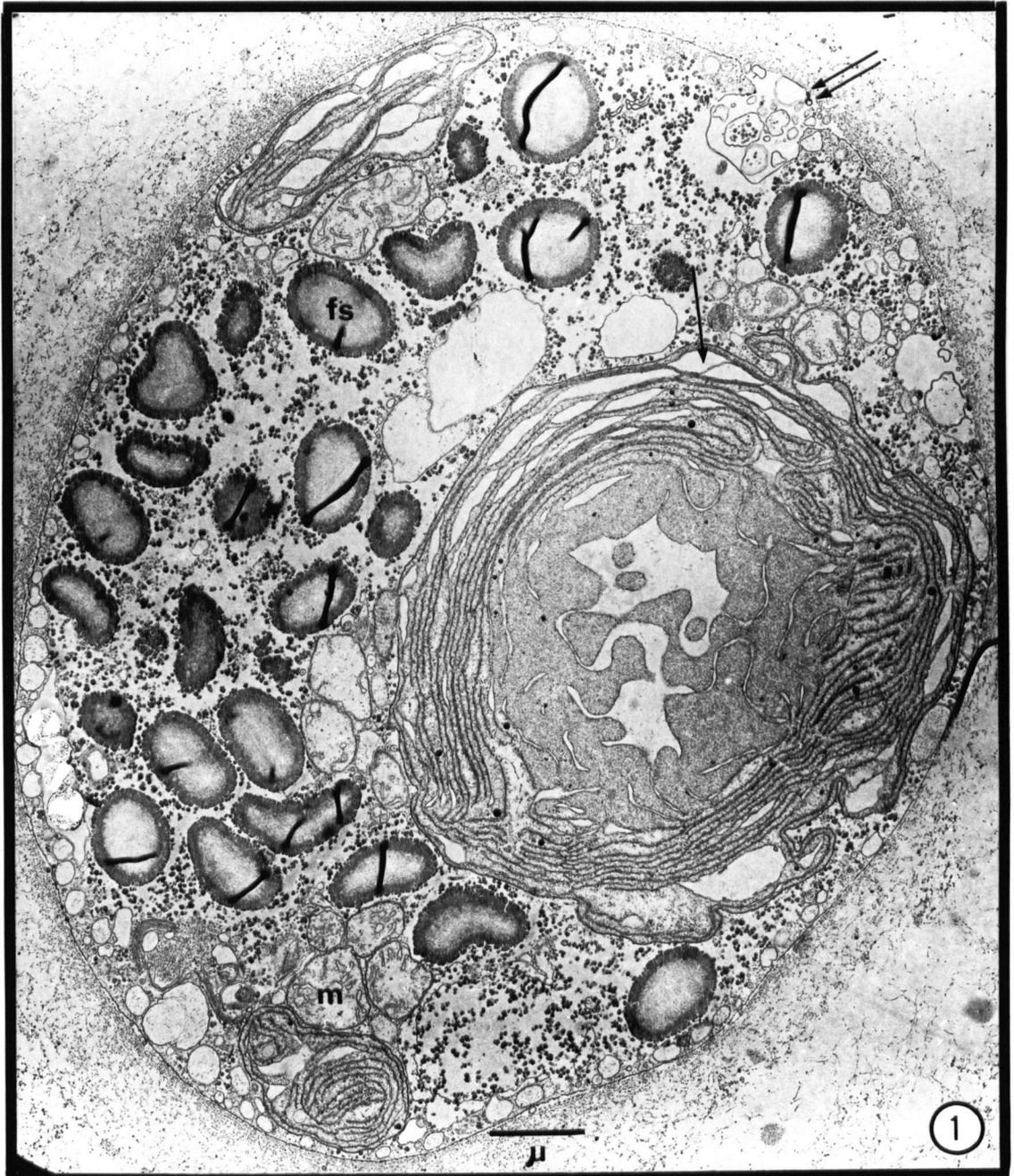


PLATE XXVIII

Smithora. Degenerating monospore

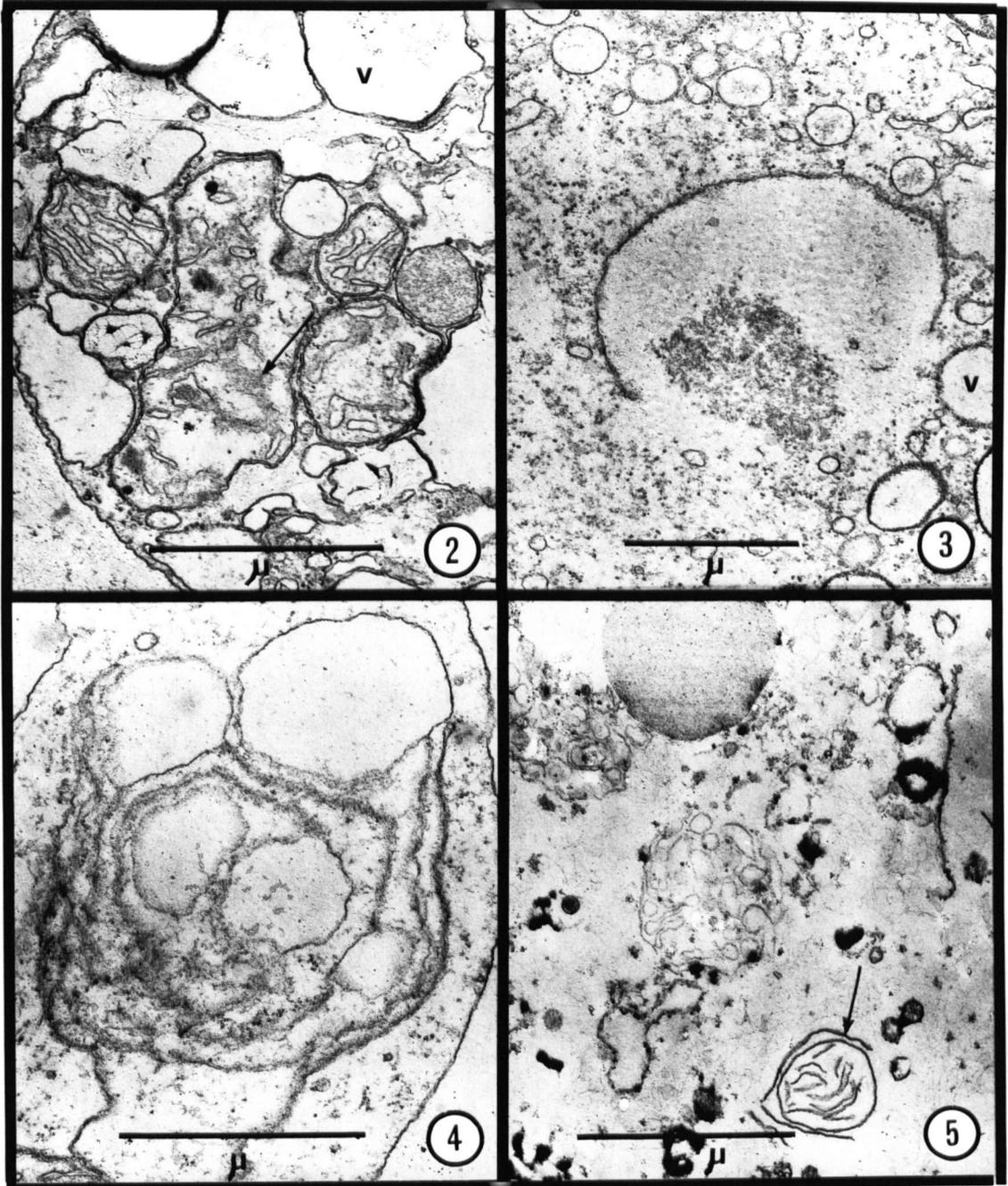
Fig. 2. Swollen mitochondria with indistinct cristae (arrow). Note numerous vacuoles (v).

Fig. 3. Nuclear breakdown indicating advanced spore degeneration. Note numerous vacuoles (v).

Fig. 4. Disorganization and swelling of chloroplast lamellae.

Fig. 5. Cellular disintegration after plasmalemma breakage. Note remaining membranous elements (arrow).

XXVIII



b) Monospore Germination in Smithora.¹

Introduction.

Studies on the ultrastructural events during algal spore germination have been relatively few in number. Although there appears to be no published investigations of this nature dealing with the Rhodophyceae, detailed studies have been presented on zoospore germination in the green algae Stigeoclonium (Manton, 1964) and Enteromorpha (Evans and Christie, 1970). Work is also being carried out on zoospore germination in Oedogonium (Chlorophyceae) (Pickett-Heaps, 1971) and recently Quatrano (1972) has investigated zygote germination in Fucus (Phaeophyceae).

Smithora provides an ideal material for such research as large numbers of spores will germinate in relative synchrony under laboratory conditions.

Observations.

At a light microscopic level, monospore germination is marked by the appearance of a cell wall and several vacuoles (Pl. XXIX, Fig. 1). At an ultrastructural level, the germinating spore also possesses a pyrenoid-containing chloroplast (Pl. XXIX, Fig. 2; Pl. XXX, Fig. 4), a nucleus (Pl. XXXI, Fig. 7,8; Pl. XXXIV, Fig. 14), dictyosomes (Pl. XXXI, Fig. 6; Pl. XXXII, Fig. 9), endoplasmic reticulum (Pl. XXXI, Fig. 7; Pl. XXXII, Fig. 9,10), mitochondria (Pl. XXIX, Fig. 2; Pl. XXXI, Fig. 7; Pl. XXXII, Fig. 10; Pl. XXXIV, Fig. 14) and some floridean starch (Pl. XXXIV, Fig. 14).

The two methods of basal holdfast formation are evident under the light microscope as well as the electron microscope. A single spore forms a cell wall (Pl. XXIX, Fig. 1,2) and through successive cell divisions produces a basal holdfast (Pl. XXXI, Fig. 5). Alternatively, an identical

¹This section is based on an article by D.L. McBride and K. Cole in Phycologia 11: 181-191 (1972).

holdfast may be formed by many spores adhering and secreting individual cell walls which eventually contribute to a common cell wall (Pl. XXX, Fig. 3,4). Wall construction appears to be initiated in the area of the cell adjacent to the peripherally situated nucleus (Pl. XXXI, Fig. 7), but eventually it is formed continuously around the cell (although there may be unrelated, localized areas of different thicknesses) (Pl. XXXI, Fig. 8). The bonding of adjacent cell walls is suggested by the observation that folds occur between thin sectioned cells only in areas where the walls are not closely interwoven (Pl. XXX, Fig. 4; Pl. XXXI, Fig. 8).

The germinating monospore is distinguished by a large increase in the peripheral ER which appears to be extremely active (Pl. XXXII, Fig. 9). Cisternae are often swollen, associated with large numbers of ribosomes and found in close proximity to the convoluted plasmalemma (Pl. XXXII, Fig. 10). Dictyosomes may also be present in these areas (Pl. XXXII, Fig. 9). It is possible that both the ER and the dictyosomes are involved in the synthesis of wall material which is a primary activity of the cell at this time.

Dictyosomes also appear to play an important role in vacuole formation. A large number of these organelles are situated with their maturing faces and associated vesicles in close proximity to the enlarging vacuoles (Pl. XXXI, Fig. 6). An association between dictyosomes and vacuoles has also been noted in the red alga Batrachospermum (Brown and Weier, 1970).

The lobed, pyrenoid-containing chloroplast is structurally unchanged from that reported in the newly released monospore (Section Va) in possessing areas of closely appressed lamellae (Pl. XXIX, Fig. 2; Pl. XXXIV, Fig. 14). However, the number of such areas appears to be somewhat reduced.

Typical floridean starch grains are conspicuously few in number.

Conditions of laboratory culture may have some bearing on the cellular content of floridean starch (Burton, H., personal communication). It is also conceivable that the amount of reserve substance may be dependent on the particular metabolic state of the cell.

Two types of pyrenoid structure are represented in germinating monospore material. A typical, centrally located pyrenoid possessing irregular traversing lamellae is the most regular feature (Pl. XXIX, Fig. 2; Pl. XXX, Fig. 4). However, a small percentage of cells exhibit a definite crystallization of the pyrenoids. These "pyrenoids" are somewhat reduced in size and many appear to have cleaved, resulting in two or three angular crystalline bodies (Pl. XXXII, Fig. 11). The crystal lattice is formed from linked subunits which appear in a parallel-line or in a cross-hatched pattern depending upon the plane of sectioning (Pl. XXXIII, Fig. 12). The parallel lines of both configurations have a centre to centre spacing of 12.5 nm. with each subunit measuring 6.0-7.0 nm. in diameter. The angle of intersection in the cross-hatched pattern is 70-75°. In addition, electron translucent areas, probably representing included traversing lamellae and the ribosome-like particles referred to by Holdsworth (1968) were observed (Pl. XXXIII, Fig. 12).

The nucleus is eukaryotic in appearance and is surrounded by numerous cisternae of rough endoplasmic reticulum (Pl. XXXIV, Fig. 14). No connections between the nuclear envelope and surrounding complement of ER were noted. Spindle fibres were occasionally observed in nuclear areas preparing for karyokinesis (Pl. XXXIV, Fig. 17). Unfortunately the microtubular elements were not preserved well under the fixation conditions employed. The fibres appear to originate externally to the nuclear envelope and radiate towards the nucleus from a spindle organizing centre. Areas adjacent to the nuclear

envelope which often appear devoid of structured material could also be centres of spindle fibre organization which have been destroyed by fixation procedures (Pl. XXXIV, Fig. 15). Bordering such areas is a high concentration of nuclear pores indicating a possible nuclear polarity at this early stage of division. La Cour and Wells (1972) have shown a more irregular distribution of pores in prophase as compared to interphase stages of certain higher plants.

Two types of vesicular units of unknown function were observed regularly in the cytoplasm of the germinating monospore. The first type is invariably associated in groups with the ER surrounding the nucleus (Pl. XXXIV, Fig. 14). Each unit is spherical or slightly ellipsoidal in shape and measures .05-.07 microns in diameter (Pl. XXXIV, Fig. 13). These units appear to consist of several concentric, membranous spheres bounding a central more electron transparent area. The second type of vesicle is a spherical, membrane bound structure which exists in large aggregations in the cytoplasm (Pl. XXXIV, Fig. 15). These eccentrically located structures superficially resemble dictyosome vesicles but are seldom seen in the vicinity of these organelles. Paramural bodies (Marchant and Robards, 1968) are few in number (Pl. XXXIV, Fig. 16) and are unlike the above mentioned vesicular units which are more regular in structure.

Discussion.

From observations on the methods of holdfast formation in Smithora it is evident that within a population of released monospores a certain number will begin development from unicellular stages while the remainder will begin development from multicellular stages of varying degrees of complexity. This mechanism could aid in desynchronizing the development of a generation of monospores. Thus, the population would reach reproductive maturity over a longer period of time, tending to minimize the possibility

of adverse environmental conditions destroying a "crop" of released monospores. Another possible advantage of the development of a holdfast from numerous spores would be a decrease in the time required to complete an asexual reproductive cycle. However, this mechanism would also reduce the number of plants developing from a given number of spores.

The nature of wall production in algal cells has been investigated by a number of authors including Brown (1969), Brown et al. (1970), Jordan (1970), Pickett-Heaps (1971) and Pickett-Heaps and Fowke (1970). In these and other studies the dictyosome has been implicated in the production of wall material or wall precursors. Positive cytochemical tests have been obtained for the presence of polysaccharides in these organelles. In addition, Thompson and Preston (1968) have suggested that proteins may be an important structural component in certain algal cell walls. Thus, one might expect a germinating spore to be actively involved in the production of proteins with enzymatic or structural functions as well as the carbohydrate moiety. Indeed, Quatrano (1968, 1972) has shown that rhizoid formation in Fucus zygotes is dependent on protein synthesis. In germinating monospores of Smithora, peripherally located rough ER appears to become associated with the plasmalemma. A similar peripherally located network has also been reported in Porphyridium (Gantt and Conti, 1965) and in Rhodella (Evans, 1970). In Smithora it appears that material could be transferred directly from the ER across the plasmalemma into the cell wall. This mechanism has also been postulated to occur in Oedogonium (Pickett-Heaps, 1971). The hypothesis that the ER acts as a final synthesis and packaging point of wall material is attractive but must be based on further studies.

In the germinating monospore the dictyosome complement is capable of carrying out several different functions simultaneously. This principle of

"division of labour" allows some of these organelles to be involved in vacuole construction while the remainder appear to take part in production of wall material. In the developing zoospore of Oedogonium Pickett-Heaps (1971) also noted two distinct populations of dictyosomes, each apparently with a different function. In this context, it is also interesting to note a change in roles of the dictyosome populations in a monospore from the differentiating state, through the free-floating condition to germination. As spore differentiation occurs the dictyosomes are concerned with the manufacture of large deposits of a material probably of a mucilaginous nature (Section Va). As the spore nears the time of release from the thallus and for a short period after, the production of an adhesive material occurs (Section Va). Prior to settling, the dictyosome populations are less active, but upon germination appear to become involved in vacuole formation and production of a wall material. Evans and Christie (1970) reported a similar set of changes in the germinating zoospore of Enteromorpha.

The crystalline matrix is an interesting, if not regular, feature of the pyrenoid. Crystalline pyrenoids have been reported previously in brown algae (Evans, 1966), diatoms (Holdsworth, 1968; Coombs et al., 1968; Taylor, 1972), a dinoflagellate (Kowallik, 1969) and a green alga (Bertagnolli and Nadakavukaren, 1970). From these studies it is evident that a crystalline matrix is not present in all pyrenoids of a particular algal population or, if present, may be apparent in particular regions only. In fact, one report details this structural property in the diatom Navicula only after colchicine treatment (Coombs et al., 1968). In this laboratory, studies on freshly collected vegetative material, monosporogenous material and spermatangial sori have shown no evidence of crystalline pyrenoids. However, in the present study monospores subjected to culture conditions have this

structural property. It is interesting to note that other illustrated reports have also been made using cultured material.

Holdsworth (1971) has recently reported the presence of proteinaceous components in the pyrenoid of Eremosphaera (Chlorophyceae) which appear to have similar properties to certain enzymes in the carbon fixation pathway of photosynthesis. Thus, it might be of interest to note the structure of pyrenoids possessing crystalline matrices under different photosynthetic conditions. Such controlled experiments may shed some light on the formation of these structures. Perhaps this phenomenon is a modification preparatory to withstanding adverse environmental conditions, or even one of the first steps in cell degeneration. Indeed, Ragetli, Weintraub and Lo (1970) have described "pseudocrystalline" structures in the chloroplasts of excised degenerating leaf material of Nicotiana which are very similar to the crystallized pyrenoid of Smithora. They suggest this may represent a highly ordered mechanism for coping with starvation.

The substructure of the crystal lattice in Smithora monospores is comparable to that reported in the diatom Achnanthes (Holdsworth, 1968) and the green unicell Chlorella (Bertagnolli and Nadakavukaren, 1970). However, the mean centre to centre distance between parallel subunit rows is different. In both the aforementioned algae this spacing was found to be 8.0 nm. while in Smithora the distance is 12.5 nm. In the dinoflagellate Prorocentrum, Kowallik (1969) reported a distance of 12.2 nm. between the centres of adjacent subunits. The significance of these differences is not yet clear although it could indicate important variations in pyrenoid composition between certain algae.

Until recently, microtubular spindle fibres have not been demonstrated in red algae (Hommersand and Searles, 1971). But now reports of these

structures by Chapman, Chapman and Lang (1971) in Porphyridium and MacDonald (J. Phycol. in press) in Membranoptera indicate that they are probably involved in cell division in this group. Their presence in Smithora further strengthens this view. However, it is evident that much more study is needed to fully elucidate this process of cell division.

In conclusion there are several fine structural changes early in the germination of the monospore which appear to be typical of this process. There is a marked reduction in floridean starch content, a notable increase in peripheral rough ER and an increase in vacuolar area. It is conceivable that these changes are integrally associated with the process of cell wall construction.

PLATE XXIX.

Smithora. Germinating monospore

Fig. 1. Light micrograph of a germinating monospore.

Fig. 2. Electron micrograph of a germinating monospore illustrating a cell wall (cw), vacuoles (v), a pyrenoid (p) and chloroplast lobes (cl). Arrow indicates area of appressed chloroplast lamellae.

XXIX

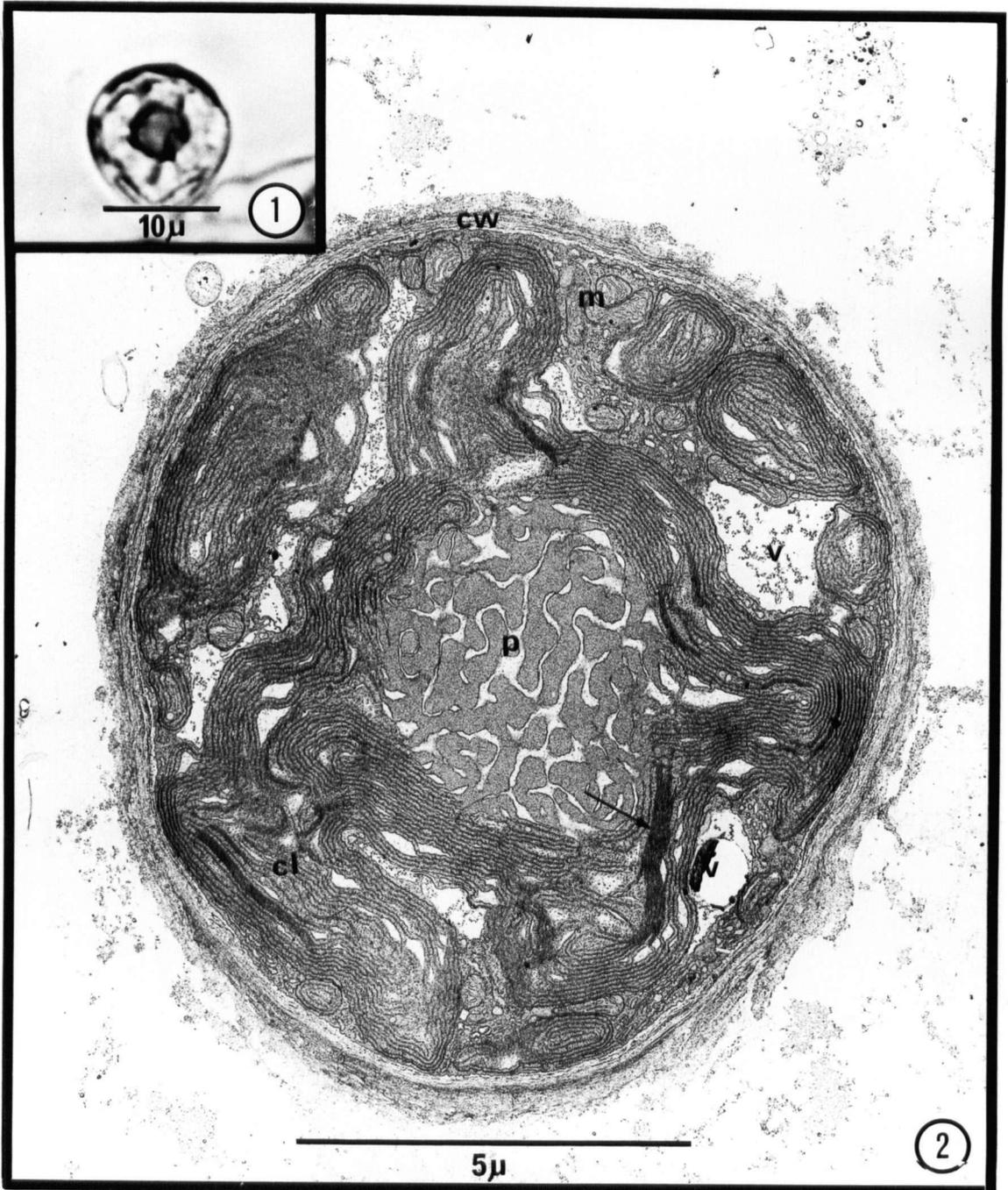


PLATE XXX

Smithora. Germinating monospore

Fig. 3. Light micrograph of a number of adhering, germinating monospores.

Fig. 4. Electron micrograph of a developing basal holdfast formed from numerous spores. Arrows indicate an area of common cell wall between two spores. Note that dark fold lines do not pass between joined spores.

XXX

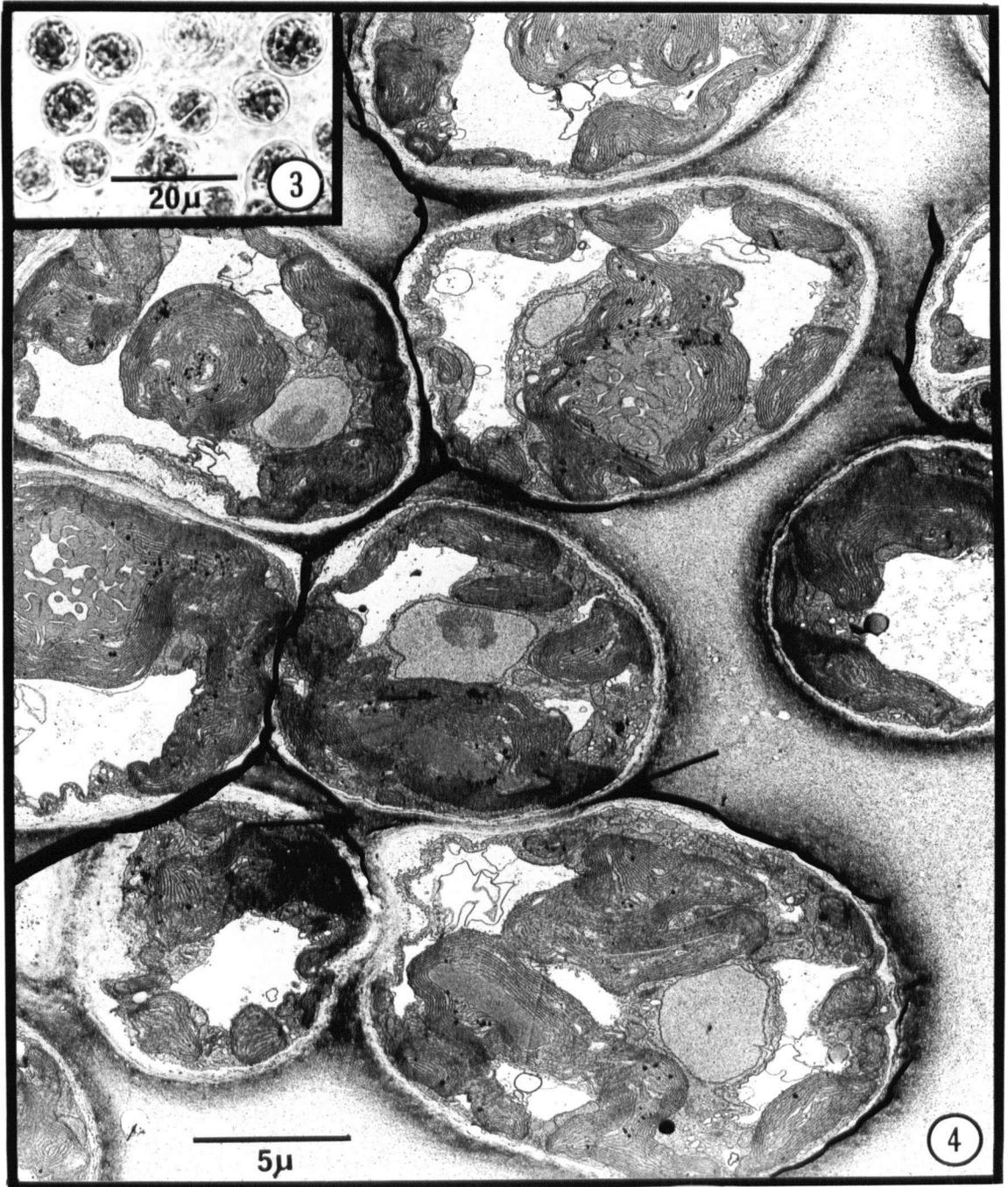


PLATE XXXI

Smithora. Germinating monospore

Fig. 5. Light micrograph of a mature basal holdfast (bh) attached to a host plant (h).

Fig. 6. A dictyosome (d) with associated vesicles in close proximity to an enlarging vacuole (v).

Fig. 7. Portion of germinating spore illustrating area of cell wall initiation (cw) adjacent to nucleus (n).

Fig. 8. Portion of a developing pad illustrating a nucleus (n), common cell wall (cw) and a vacuole (v). Arrow indicates an area of thickening cell wall.

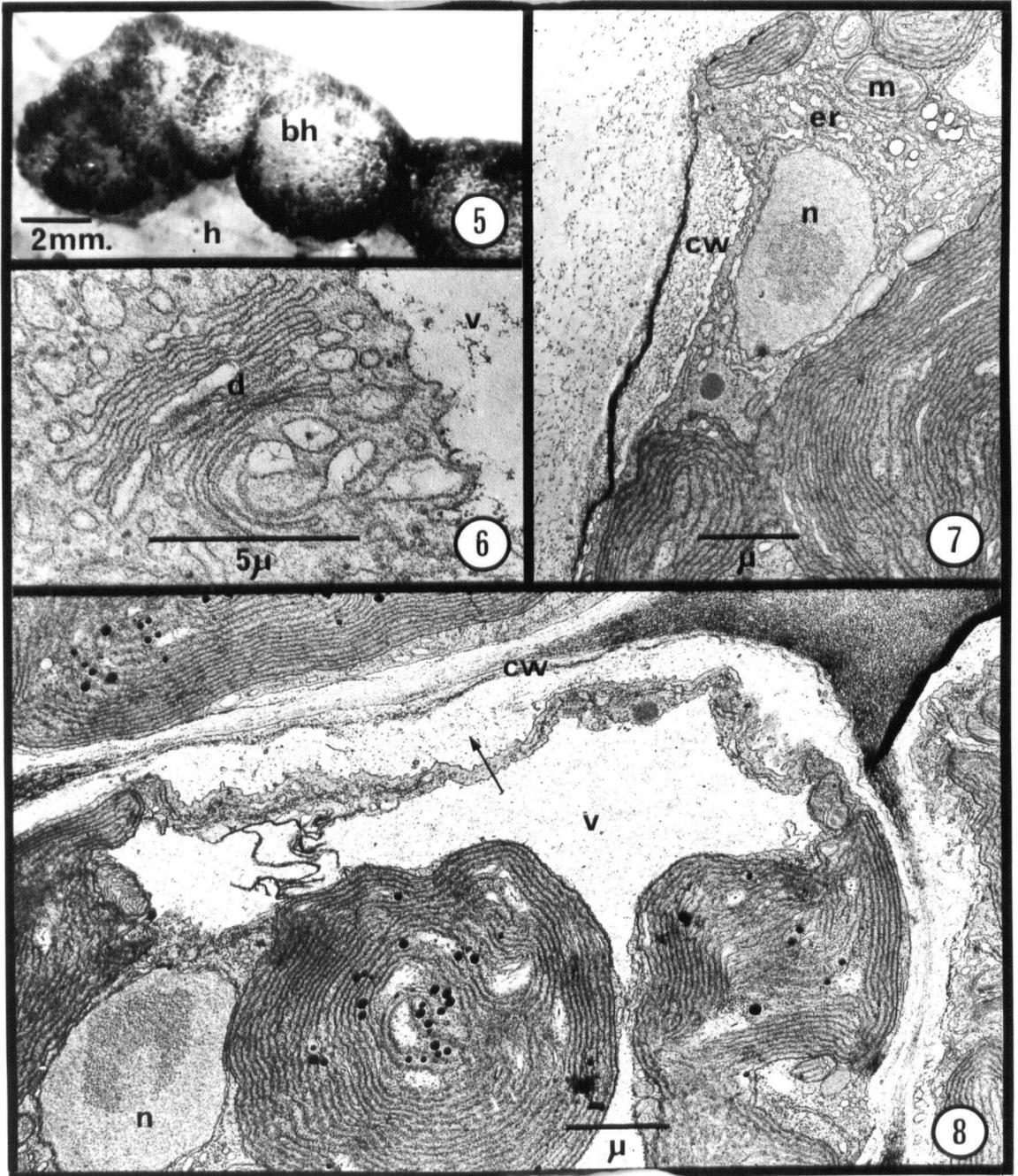


PLATE XXXII

Smithora. Germinating monospore

Fig. 9. A tangential section through a portion of a monospore showing hypertrophied peripheral ER and dictyosomes (d).

Fig. 10. Rough ER with cisternae in close association with the plasmalemma. A mitochondrion (m), a vacuole (v) and the cell wall are also labelled.

Fig. 11. A portion of a chloroplast (c) showing single lamellae (arrow) and a cleaved, angular crystalline pyrenoid (cp).

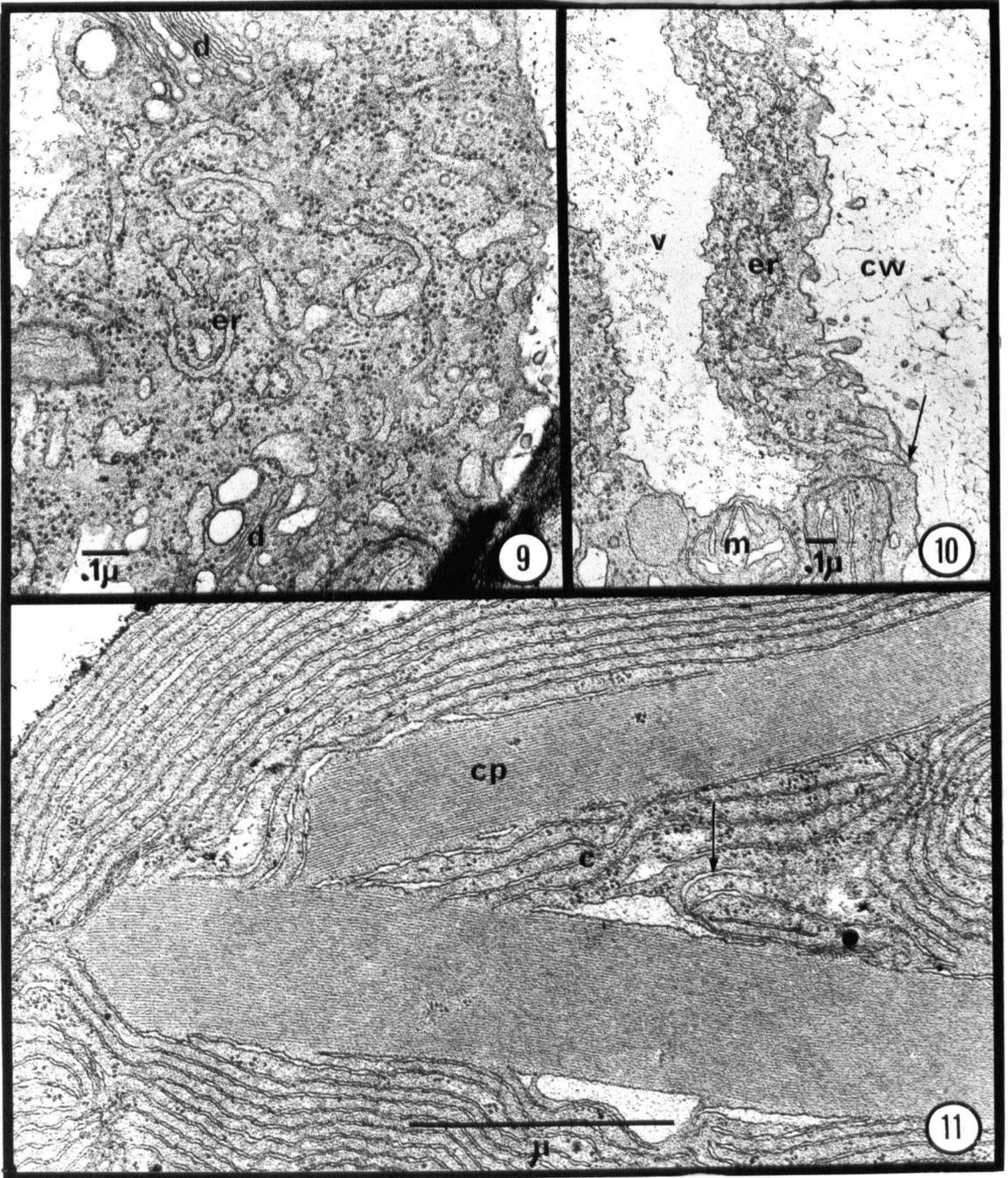


PLATE XXXIII

Smithora. Germinating monospore

Fig. 12. Section through a crystalline pyrenoid illustrating the nature of the crystal lattice. Note the included traversing lamella (tl) and ribosome-like particles (arrow).

XXXIII

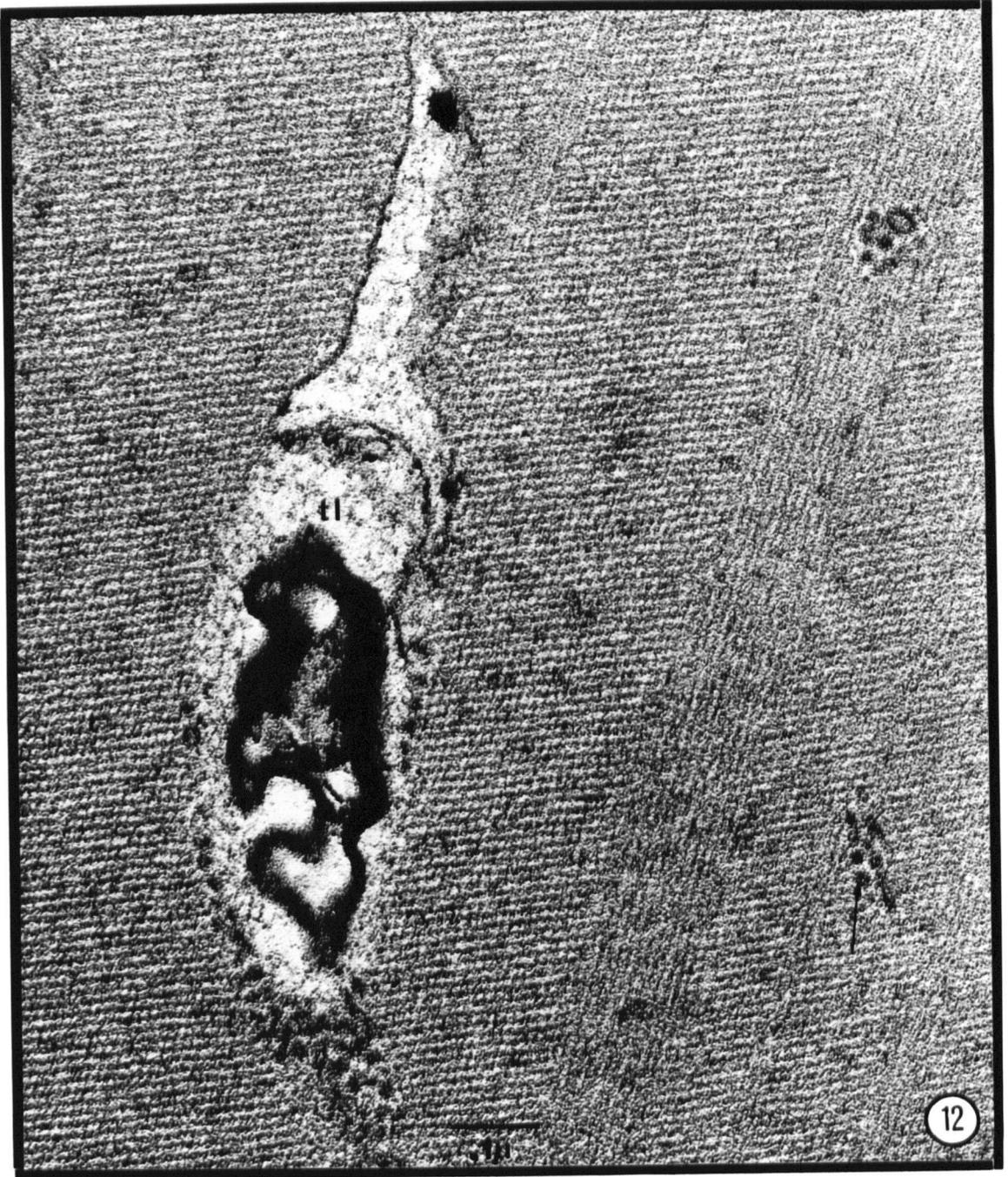


PLATE XXXIV

Smithora. Germinating monospore

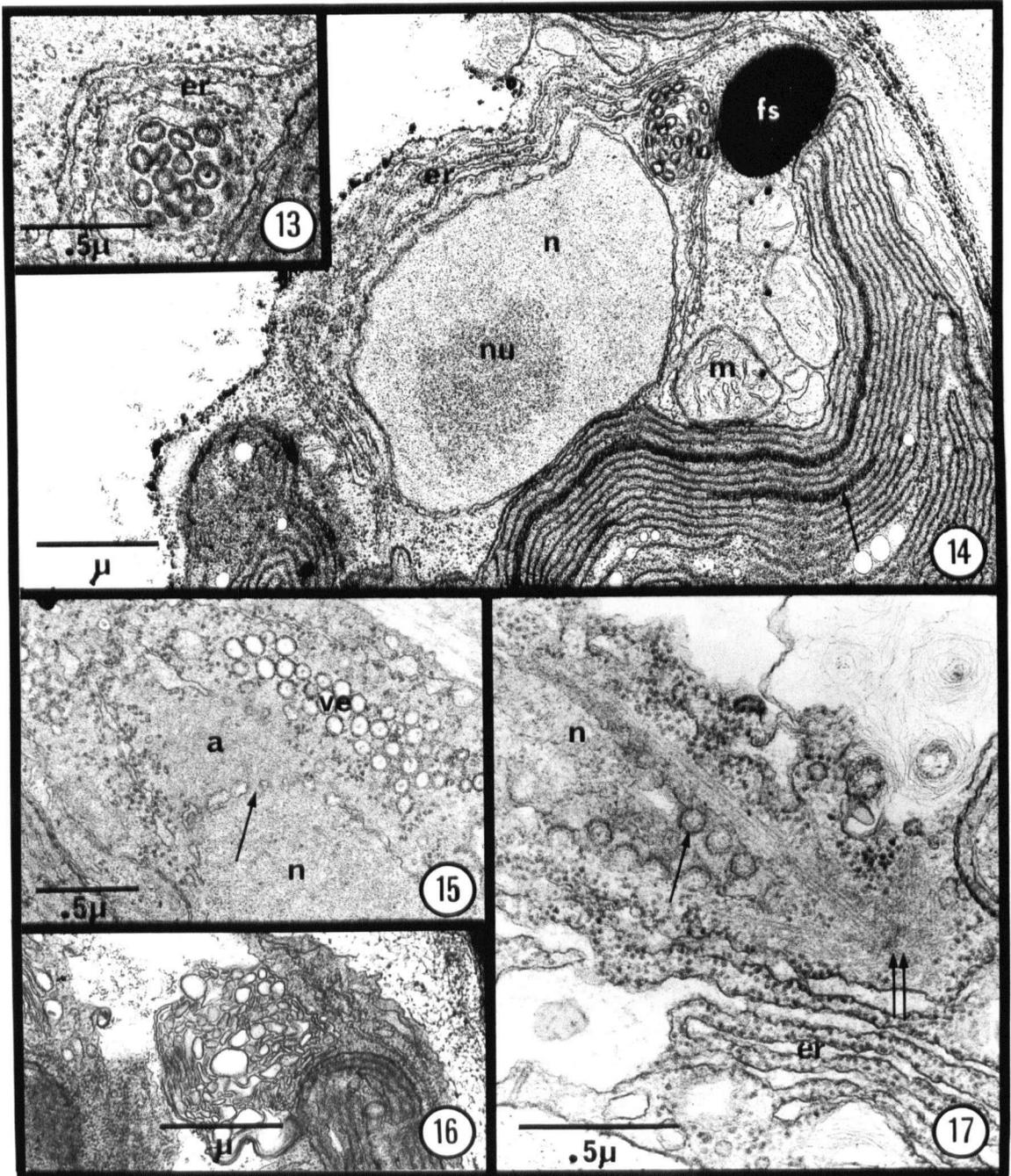
Fig. 13. A group of vesicular units in association with the ER.

Fig. 14. Portion of a cell showing the location of the vesicular units near the nucleus (n). A nucleolus (nu), ER, a floridean starch grain (fs) and appressed chloroplast lamellae (arrow) are also marked.

Fig. 15. Section through a nuclear area (n) showing an aggregation of vesicles (ve). Note the unstructured area of cytoplasm (a) adjacent to the porous nuclear envelope (arrow).

Fig. 16. Paramural body near cell wall.

Fig. 17. Microtubular spindle fibres radiating toward the nucleus (n). Note the tangential sections of nuclear pores (single arrow), ER, and the extranuclear location of the spindle fibre organizing centre (double arrow).



VI. ULTRASTRUCTURAL EVIDENCE OF SEXUAL REPRODUCTION

Introduction.

Perhaps one of the most interesting and important aspects of studies on red algae are the descriptions of sexual reproduction. Although this process is well documented in many members of the Florideophycidae, it is poorly known in most Bangiophycidae. During this study, the only evidence of sexual reproduction in the Erythropeltidaceae was the regular production of "spermatia" (Hollenberg, 1959) in Smithora and sporadic indications of fertilization in Erythrotrichia boryana. It must be stressed that in this report terms describing sexual reproduction (e.g. spermatia, carpospore, etc.) are used in their most tentative sense due to the lack of information on their specific function in this family. As is shown by the various conflicting reports of sexual reproduction in the Erythropeltidaceae, the size of the plants and, more specifically, the unicellular nature of the reproductive "organs" hinders documentation using conventional light microscopic techniques. On the other hand, the seemingly transient nature of this process (fertilization, in particular) provides a formidable barrier to electron microscopic investigation. In addition, many such algae tend to display only asexual reproduction in laboratory conditions. These are indeed perplexing problems which have undoubtedly caused some of the present confusion surrounding sexual reproduction in these plants.

Observations.

Spermatiaogenesis in Smithora: Spermatia may be produced extensively in the medial distromatic portion of the mature blade in the fall of the year. At a light microscopic level, each vegetative cell appears to undergo an asymmetric division parallel to the surface of the thallus, resulting in the production of numerous, small (3-5 microns), pale cells (Pl. XXXV,

Fig. 1).

At an ultrastructural level, initiation of this process involves the migration of the usually eccentrically located nucleus toward the end of the cell nearest to the surface of the thallus (Pl. XXXV, Fig. 2), allowing the formation of unequal daughter cells. After division it appears that the nucleus of the larger cell migrates toward the opposite end of the protoplast (Pl. XXXV, Fig. 2).

The newly formed spermatangium is not only smaller than the vegetative cell but contains little chloroplast material (contrary to Hollenberg's (1959) light microscope observation) and no pyrenoid (Pl. XXXVI, Fig. 3). Evidently only one or two chloroplast lobes have been included during the unequal division. However, a typical nucleus (Pl. XXXV, Fig. 2; Pl. XXXVI, Fig. 3,5), mitochondria (Pl. XXXVI, Fig. 3; Pl. XXXVII, Fig. 8), ER (Pl. XXXVI, Fig. 3), dictyosomes (Pl. XXXVI, Fig. 4,5), floridean starch grains (Pl. XXXVI, Fig. 3; Pl. XXXVII, Fig. 8) and perhaps a few small vacuoles (Pl. XXXVI, Fig. 3) are usual components of the spermatangium.

Dictyosomes appear to play an important role in the maturation of these structures. Soon after cell division these organelles begin to hypertrophy (Pl. XXXVI, Fig. 4) and produce numerous vesicles containing a fibrillar substance (Pl. XXXVI, Fig. 5) reminiscent of that in the differentiating monospore (Section Va). However, the contents of the spermatangial vesicles appear more compacted with a central, electron dense core of highly compressed fibrils (Pl. XXXVI, Fig. 5; Pl. XXXVII, Fig. 6,7). These structures are then released into the cell wall by a process similar to reverse pinocytosis involving a fusion of the vesicular membrane with the plasmalemma (Pl. XXXVII, Fig. 6). The material accumulates between the spermatangial protoplast and the cell wall, particularly at the margin nearest the plane of the

thallus (Pl. XXXVII, Fig. 6,7). Thus, the surface of a mature sorus exhibits many irregular protrusions of cell wall material at sites of spermatogenesis (Pl. XXXV, Fig. 2; Pl. XXXVII, Fig. 7). Eventually the cell wall ruptures and the naked spermatium is released (Pl. XXXVIII, Fig. 9,10). At this point it is evident that the material produced by the dictyosomes is liquid or semiliquid in nature as a large amount appears to flow from the thallus after cell liberation.

Under the light microscope the released spermatium is almost colourless due to the lack of extensive chloroplast material (Pl. XXXVIII, Fig. 11). It is ultrastructurally identical to the unreleased cell with a certain amount of vesicular material appearing to remain after release (Pl. XXXVIII, Fig. 9). Numerous attempts to document their function were unfruitful.

Evidence of fertilization in *Erythrotrichia boryana*: The presence of "spermata" adhering to filaments of *E. boryana* was noted under the light microscope during two separate collections in the fall of 1971. These attached structures are virtually colourless and possess a cell wall (Pl. XXXIX, Fig. 1). It is evident that the spermatium did not preserve well under the fixation procedure used for electron microscopy (Pl. XXXIX, Fig. 4). However, considering the nature of the loosely organized cell wall and the presence of typical floridean starch granules, these pale cells are certainly red algal and most probably originated in different filaments of *E. boryana*. Mitochondria, dictyosomes and vacuoles are additional discernible structures (Pl. XXXIX, Fig. 4). No nuclear material was observed but this may have been destroyed by fixation procedures. Of interest is the short cellular "stalk" or "foot" present in the area of attachment (Pl. XXXIX, Fig. 1,2,4). Directly beneath this point, the underlying cell usually appears to have undergone an unequal division similar to that in the spermatangial sorus of *Smithora*,

with the resulting small cell being virtually identical to the spermatangium. This "carpospore-like" cell contains a nucleus, a small amount of chloroplast material, mitochondria, dictyosomes, ER and floridean starch grains (Pl. XXXIX, Fig. 3). No subsequent stages of vesicle production were noted although this could be due to the particular stage of development at the time of observation. No trichogyne-like structures or intercellular connections of any type were observed.

Discussion.

It is apparent that the release of dictyosome originated vesicles into the cell wall is an integral part of the mechanism of spermatial liberation in Smithora. When a large amount of this mucilage-like material is accumulated external to the protoplast, the wall appears to burst as if due to physical pressure. It may also be possible that this material has some effect on the structural stability of the wall. Mucilage-like material has also been associated with the release of spermatia in other red algae (Neushul, M., personal communication). This mechanism may be likened to that postulated to aid in erythropeltidacean monospore release (Section Va).

Ultrastructural observation of the mature spermatangial sorus graphically illustrates Drew's (1956) statement: "... it was not determined whether the small cell on the outside of the filament was establishing or losing contact." As is shown in Pl. XXXV (Fig. 2), after cell release a protoplasmic "finger" from the underlying vegetative cell may project into the pore giving a false but classic example of a trichogyne. If the spermatium is still near the entrance to the pore a false impression of fertilization is imparted. Thus, previous reports of fertilization in this group must be re-examined.

Since the production and release of spermatia is a regular feature of

Smithora, it is probable that they play an important role in the life cycle of the plant. These structures must carry out their function relatively rapidly due to their apparent fragility. However, they did not survive in culture, thus, it is not possible at this time to directly implicate spermatia in the sexual process of Smithora.

At a light microscope level the evidence of fertilization in E. boryana is indeed intriguing. In classical terms the observations related here would represent a situation after fertilization prior to carpospore release. The production of carospores in E. boryana would then be identical to spermatogenesis as described by Berthold (1882) in E. ciliaris and Hollenberg (1959) in Smithora. It is difficult to directly dispute this hypothesis since, in this report, a nucleus was not observed in the attached cell. However, because it otherwise appears to possess a complete set of cytoplasmic components, some mechanism would be needed to selectively release nuclear material. This is unlikely in view of the nature of cell fusion in other organisms. Because of the presence of a cell wall, it is also unlikely that the attached cell is in the process of release. The formation of a spermatial cell wall has been reported to occur in other red algae during the free-floating state or after adhering to the carpogonial area (see Fritsch, 1945 for review).

An alternative, and perhaps more attractive, explanation of the situation in E. boryana would involve the induction of female gametogenesis by the attached spermatium and subsequent fusion with the carpospore-like cell. Induction of gametogenesis, possibly the result of a chemical stimulus, has been described in other algal species (Coleman, 1962). In addition, this mechanism would require de novo synthesis of a pyrenoid which has been postulated to occur in the zoospores of the green algae Oedogonium

(Hoffman, 1968) and Tetracystis (Brown and Arnott, 1970).

If this general hypothesis were to prove tenable, this phenomenon might be classified as a type of isogamous reproduction. It is interesting to note that this category of reproduction is characteristic of simple representatives of the Chlorophyceae and the Phaeophyceae. Nevertheless, such a theory must be further substantiated before detailed presentation.

PLATE XXXV

Smithora. Spermatangia

Fig. 1. Light micrograph of a cross-section of a spermatangial sorus.

Fig. 2. Electron micrograph of a cross-section of a spermatangial sorus.

Note irregular surface of thallus, a finger-like projection of vegetative protoplasm (arrow) and the different positions of the nuclei (n).

XXXV



PLATE XXXVI

Smithora. Spermatangia

Fig. 3. Immature spermatangium illustrating small amount of chloroplast material (c), floridean starch grains (fs), ER, a nucleus (n) with nucleolus (nu), vacuoles (v) and mitochondria (m).

Fig. 4. Immature spermatangia with dictyosomes beginning to hypertrophy (arrow).

Fig. 5. Maturing spermatangium with centrally located nucleus (n) and dictyosomes (d) producing numerous vesicles containing a fibrillar substance (arrow).

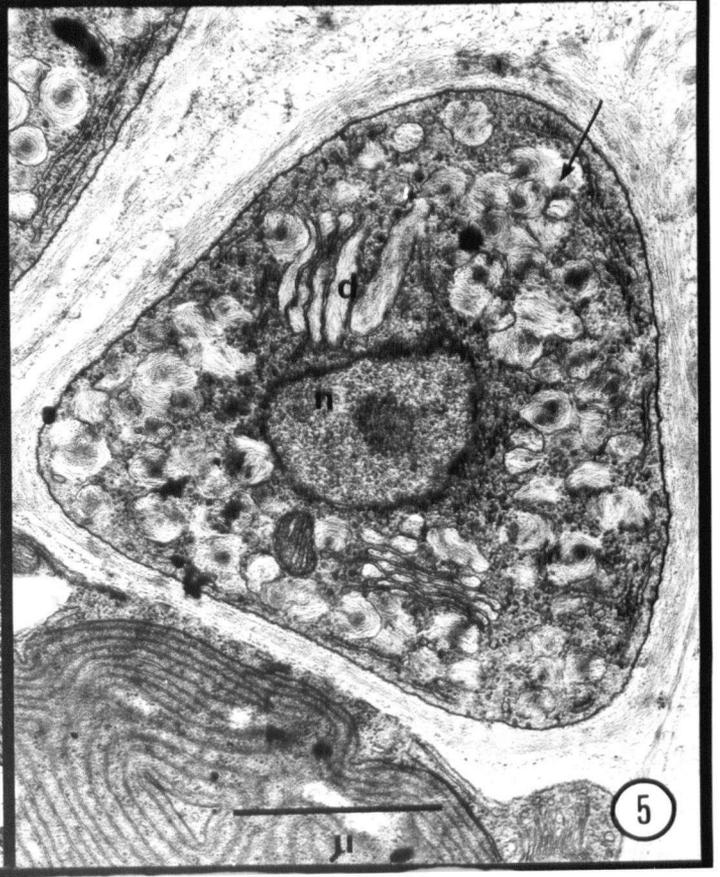
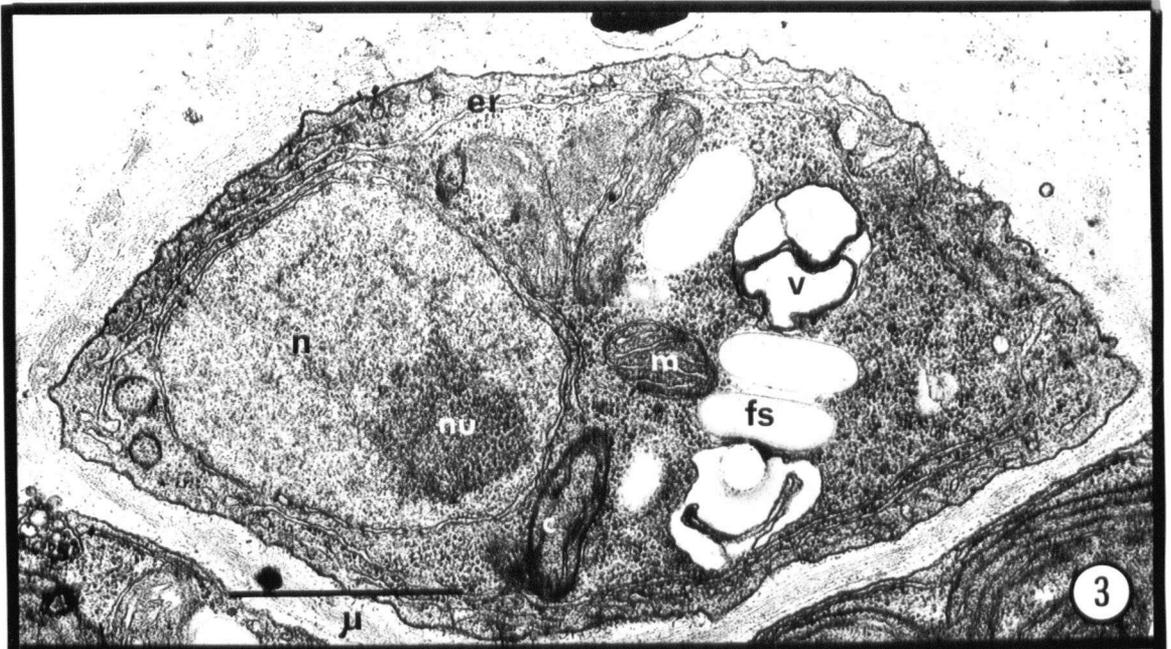


PLATE XXXVII

Smithora. Spermatangia

Fig. 6. Cell releasing contents of dictyosome derived vesicles (arrow) into cell wall. Note buildup of material around protoplast.

Fig. 7. A large quantity of the fibrillar material has been released into the cell wall. Note the protrusion of wall material in the direction of the arrow.

Fig. 8. Portion of a maturing spermatangium illustrating nature of chloroplast material (c). A mitochondrion (m) and the nucleus (n) are also marked.

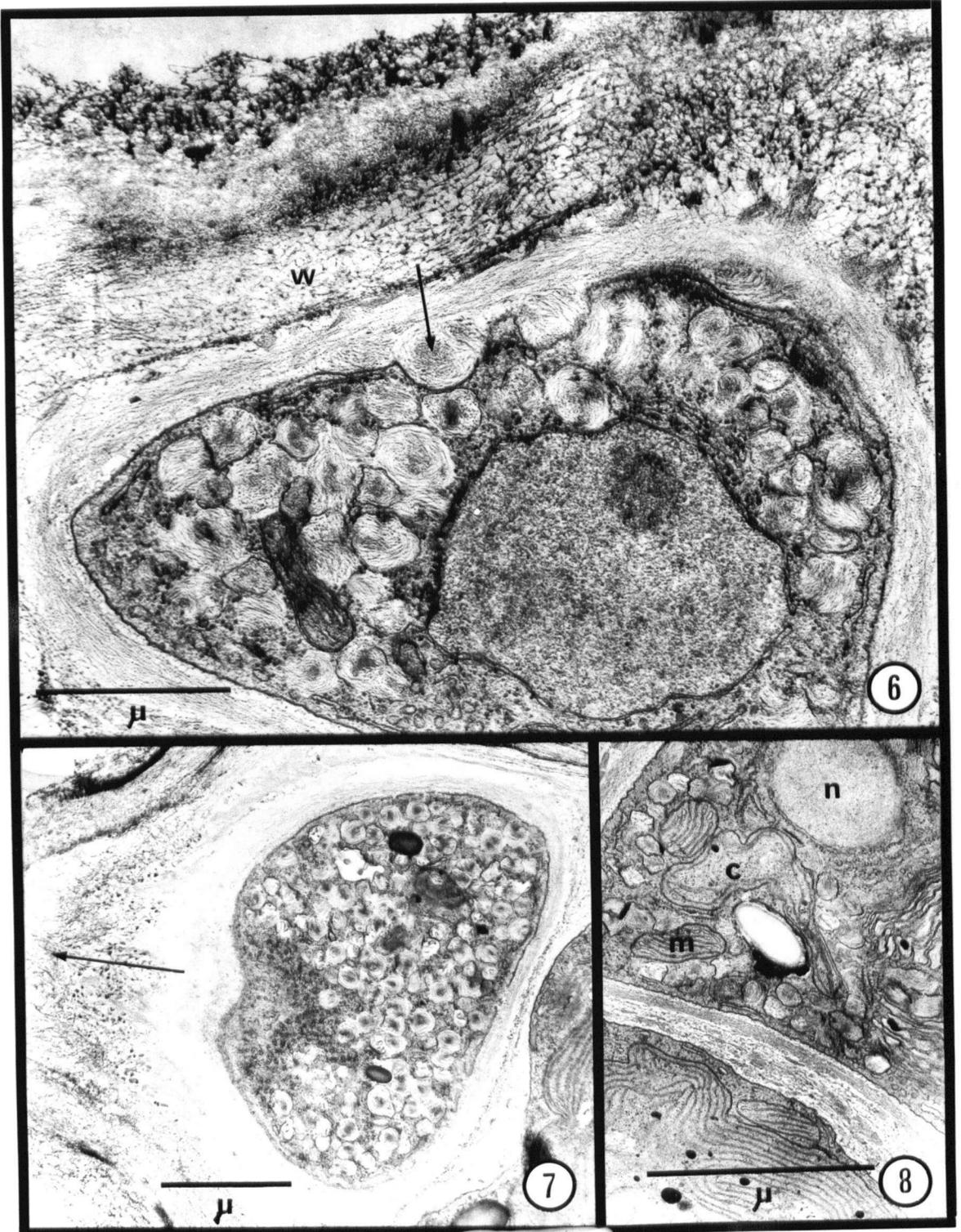


PLATE XXXVIII

Smithora. Spermata

Fig. 9. Newly liberated, naked spermatium containing a nucleus (n), floridean starch grains (fs), ER, some chloroplast material (arrow), mitochondria (m) and some remaining fibrillar material (Double arrow). Note the nearby protoplast of the vegetative cell (vc) and the copious amount of fibrillar material between the two cells.

Fig. 10. Released, free-floating spermata.

Fig. 11. Light micrograph of released, pale spermata.

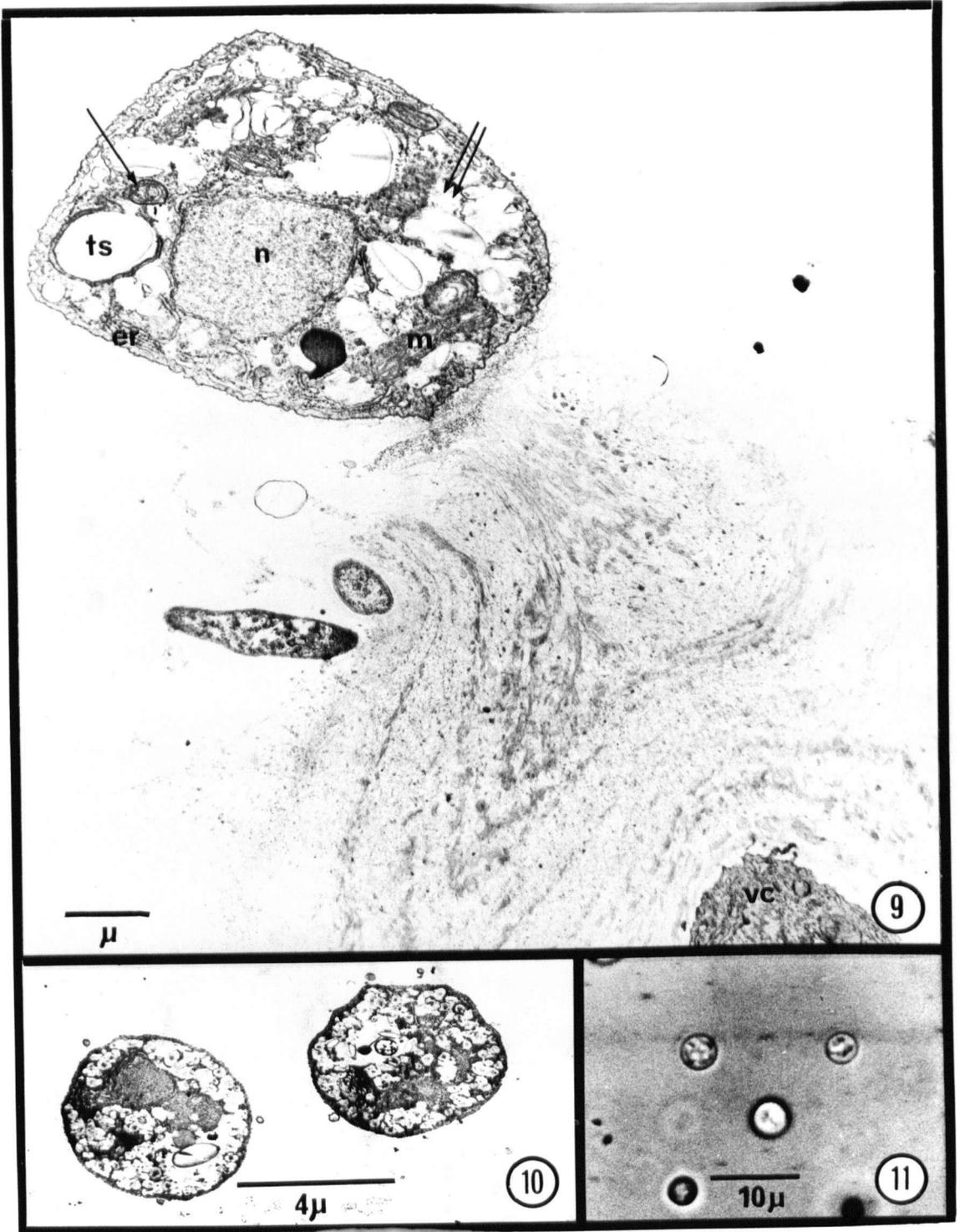


PLATE XXXIX

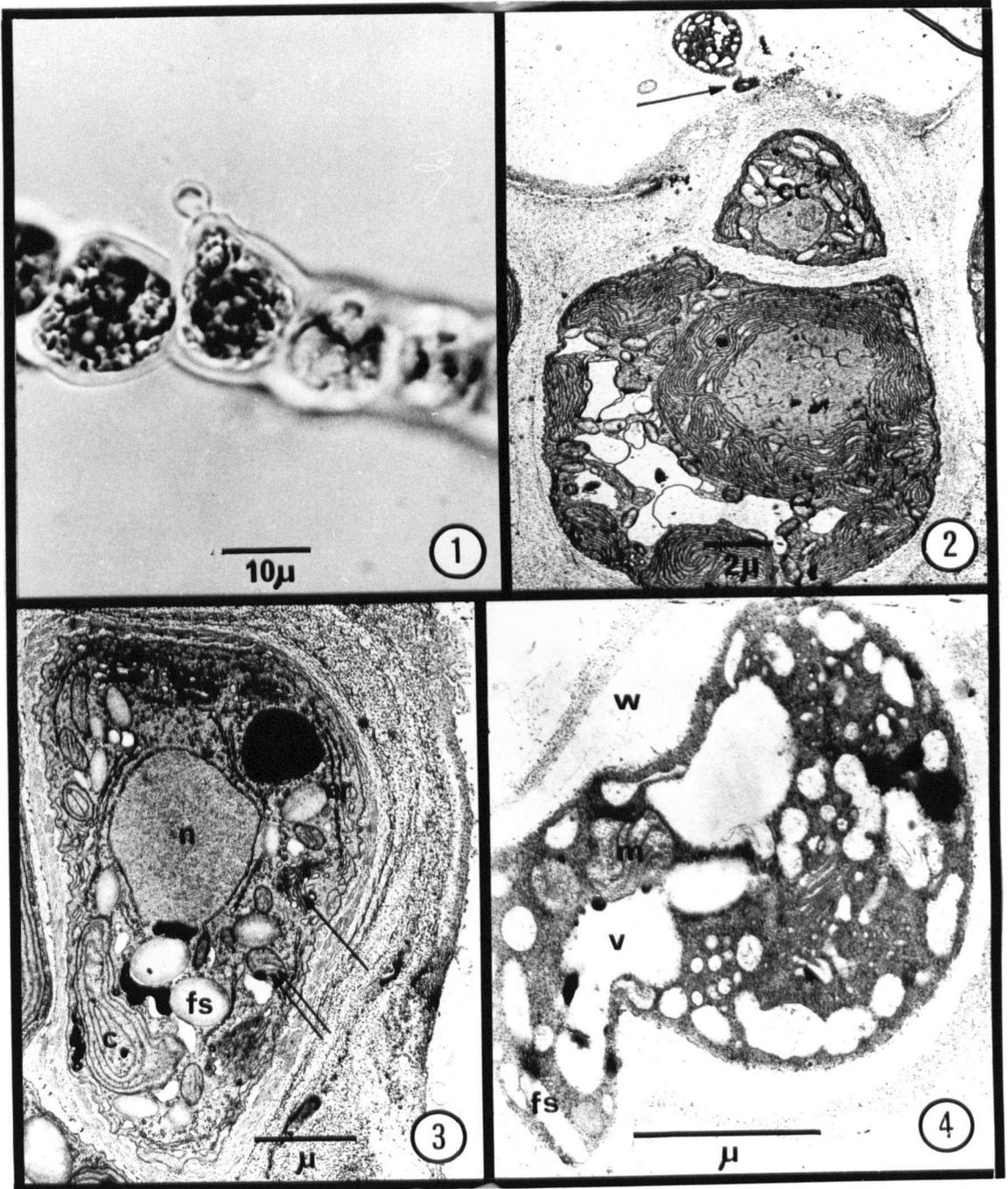
Erythrotrichia boryana.

Fig. 1. Light micrograph of spermatium attached to filament.

Fig. 2. Electron micrograph of spermatium attached to filament. Note carpospore-like cell and "foot" of attached cell.

Fig. 3. Carpospore-like cell containing a nucleus (n), chloroplast material (c), floridean starch grains (fs), ER, dictyosomes (arrow) and mitochondria (double arrow).

Fig. 4. Attached spermatium exhibiting cell wall (w), floridean starch grains (fs), a mitochondrion (m) and vacuoles (v).



VII. GENERAL DISCUSSION AND CONCLUSIONS

A proposal on the evolution of growth types in the Bangiophycidae: From ultrastructural observations on the Erythropeltidaceae reported here and from published results on other members of the subclass Bangiophycidae it is evident that there are two distinctly different types of red algal, pyrenoid-containing chloroplasts. The structural difference is best shown in a cross-section of a chloroplast lobe (Pl. XL). In the first type, many of the photosynthetic lamellae terminate at the chloroplast envelope, indicating the absence of a peripheral thylakoid. In the present discussion, chloroplasts exhibiting this structure will be designated type I. The following bangiophycean genera possess chloroplasts of this category: Porphyridium (Brody and Vatter, 1959; Speer, Dougherty and Jones, 1964; Gantt and Conti, 1965, 1966; Gantt, Edwards and Conti, 1968; Guerin-Dumartrait, Sarda and Lacourly, 1970; Neushul, 1970; Wehrmeyer, 1971; Ramus, 1972), Rhodella (Evans, 1970), Bangia (Honsell, 1963; Sommerfeld and Leeper, 1970; Cole, unpubl.), Porphyra (Gibbs, 1960; Ueda, 1961; Yokomura, 1967; Kito and Akiyama, 1968; Kazama and Fuller, 1970; Bourne, Conway and Cole, 1970; Lee and Fultz, 1970; Bourne, 1971).

Alternatively, type II chloroplasts exhibit a peripheral thylakoid and occur in the following genera: Rhodosorus (Giraud, 1963), Erythrocladia (McBride, unpubl.), Erythrotrichia (see sections IV, V, VI), Goniotrichum (McBride, unpubl.), Smithora (see sections IV, V, VI).

On the basis of this well defined difference, it is possible to construct a proposal on the evolution of growth types in the Bangiophycidae (Pl. XL).

Those multicellular genera in the "Porphyridium" line (type I) are considered to be members of the family Bangiaceae in the order Bangiales (presence of rhizoidal processes in the lower cells; carposporangia and

spermatangia producing numerous carpospores and spermatia respectively). The multicellular genera of the "Rhodosorus" line (type II) are members of the family Erythropeltidaceae in the order Bangiales or the family Goniotrichaceae in the order Goniotrichales (most lack rhizoidal processes and although knowledge of sexual reproductive processes is incomplete, where known, one spermatium is produced per spermatangium).

Unfortunately, not all Bangiophycidae can be presently included in the scheme because their chloroplast structure is unknown. It would be especially valuable to examine such rare plants as the unicellular Rhodospira and Chroothoece and the multicellular Asterocystis and Porphyropsis. Since the creation of an evolutionary theory must rely on fundamental similarities and differences, the value of the scheme as outlined would lie in the presumably very basic, genetically controlled characteristics of chloroplast structure.

The question arises as to which evolutionary line could have given rise to the Florideophycidae (if indeed only one line gave rise to this group). Evidence in favor of the Rhodosorus line is appealing. Chloroplasts of type II are found in certain members of the florideophycean order, Nemaliales e.g. Acrochaetium sp. (McBride, unpubl.) and Thorea riekei (Bischoff, 1965). Most other members of the Florideophycidae contain disc-like chloroplasts which exhibit a peripheral thylakoid. Possibly these structures could be derived more easily from a type II chloroplast. Evidence in favor of florideophycean origin from the Porphyridium line includes the presence of pit connections in the conchocelis phase of Porphyra (Bourne, Conway and Cole, 1970; Lee and Fultz, 1970) and Bangia (Sommerfeld and Leeper, 1970). However, pit-like structures are a common feature of certain Ascomycetes and have been reported in some blue-green algae

(Butler and Allsopp, 1972), suggesting that they could have arisen independently several times.

The problem of the origin of the red algae is still somewhat vexing due in most part to the lack of fossilized forms. Klein and Cronquist (1967) have reviewed the various arguments for and against a phylogenetic association between the blue-green and the red algae. A discussion of this type is beyond the scope of this report. However, it is generally agreed that the ultrastructural and physiological characteristics of the alga Cyanidium (e.g. Hirose, 1958; Allen, 1959; Rosen and Siegesmund, 1961; Mercer, Bogorad and Mullens, 1962; Seckbach, 1971) indicate an intermediate position between the two groups. In this context, it must be stressed that the evolutionary proposal presented here does not suggest direct links among the algae described. It is realised that present day forms are the result of years of evolutionary stress and selection and can be used only as examples of growth types.

Changes in the number and function of subcellular components during the course of the life cycle: Distinct changes in the number and the

amount of subcellular components were noted during the different phases in the life cycle of the plants. In some cases, concomitant with these changes, a difference in function was observed. Using the vegetative cell as a standard for comparison, these changes are outlined below:

- 1). Nucleus- there is an increase in the number of nuclear pores during monospore differentiation.
- 2). Dictyosome- these organelles hypertrophy at the onset of monospore differentiation and become involved in the formation of two products, one of which continues to be produced after spore release. Upon monospore germination these organelles are concerned with cell wall and vacuole formation.

During spermatogenesis in Smithora, dictyosomes also hypertrophy and produce a fibrillar product.

3). Endoplasmic reticulum- monospore differentiation results in an increase in perinuclear ER while subsequent spore germination is typified by a substantial increase in peripheral ER which may be involved in cell wall formation.

4). Mitochondria- these organelles increase in number during monospore differentiation. Swelling and inner membrane disruption occur during spore degeneration.

5). Chloroplast- lamellae form "pseudogranum-like" structures during monosporogenesis and swell during degeneration.

6). Vacuoles- a loss of these structures occurs during monosporogenesis with subsequent reformation upon monospore germination. A substantial increase in the number of vacuoles is noted during spore degeneration.

7). Floridean starch grains- these structures increase in number and size during monospore differentiation but undergo a rapid decrease upon monospore germination.

8). Multivesicular bodies and concentric lamellar bodies- there is an increase in the number of both these structures in older cells and a large number of the former in degenerating spores.

Conclusions.

New information has been recorded on the distribution, life histories and ultrastructure of four species of Erythropeltidaceae found in the coastal waters of British Columbia.

North American Pacific coast distribution:

Erythrotrichia carnea- Piper's Lagoon, Vancouver Is., British Columbia to Golfo Dulce, Costa Rica; Clipperton Is.

Erythrotrichia boryana- Point No Point (Glacier Pt.), Vancouver Is., British Columbia to Bahía Ascunción, Baja California, Mexico.

Erythrotrichia pulvinata- Bamfield, Vancouver Is., British Columbia to Bahía Asunción, Baja California, Mexico.

Smithora naiadum- Cape Chiniak, Kodiak Is., Alaska to Isla Magdalena, Baja California, Mexico.

Life histories:

Monospores are produced from the basal attachment organs of Erythrotrichia pulvinata and Smithora naiadum.

Ultrastructure:

Vegetative cell- A similar vegetative ultrastructure is found among the species examined. S. naiadum, E. boryana and E. pulvinata exhibit an irregular protoplast, a pyrenoid containing chloroplast with single uniform lamellae, multivesicular bodies and other typically rhodophyceae subcellular components. In addition, the chloroplast lamellae of Smithora are occasionally associated in primitive bands or stacks. E. carnea is somewhat different than the above species, notably in cell wall and pyrenoid ultrastructure.

Monospore differentiation, release and degeneration- The formation and release of monospores is dependent on the activities of the dictyosome. These organelles produce two substances, one of which may be involved in spore release, the other in spore attachment. A large amount of perinuclear ER, an increased number of mitochondria and a large number of nuclear pores are typical of the differentiating monospore. The naked, released spores possess interesting "pseudogranum-like" lamellar associations. A certain percentage of spores appear to undergo an organized degeneration in culture.

Monospore germination- In the settled monospore, the formation of a cell wall

occurs adjacent to the nuclear region. The germinating spore is typified by an increase in vacuolar area and a large amount of peripheral ER which may be involved in cell wall formation. Microtubular spindle fibres and a crystalline matrix in the pyrenoid are irregularly present in these cells. Sexual reproduction- Because gametogenesis and fertilization are poorly documented in the Erythropeltidaceae, the ultrastructural account of spermatogenesis in Smithora is of interest. The dictyosome plays an important role in the maturation of these pale cells. In addition, the ultrastructural evidence of fertilization in E. boryana suggests the possible occurrence of an induction phenomenon.

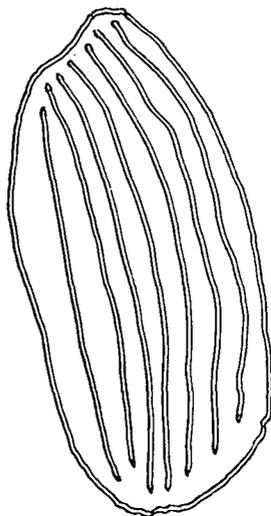
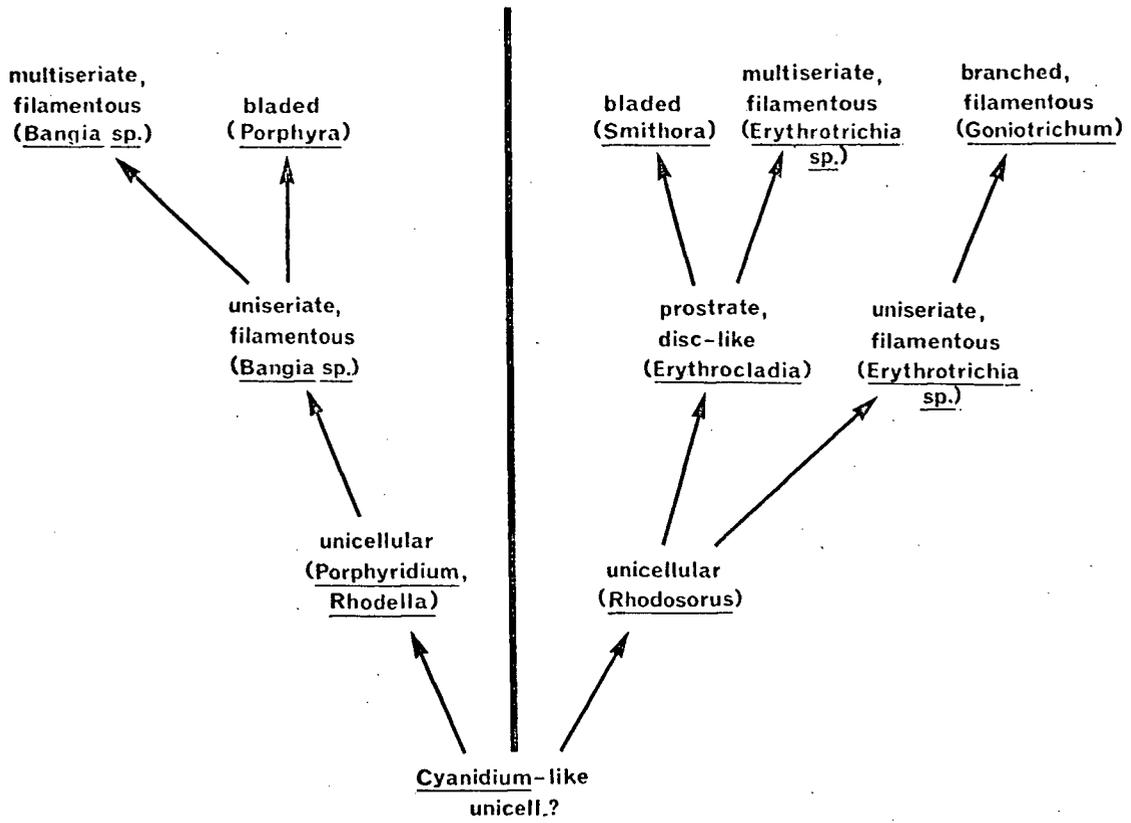
The ultrastructural characteristics of the erythropeltidacean chloroplast and other published data on chloroplast ultrastructure in the Bangiophycidae have allowed the presentation of a bilateral scheme on the evolution of growth types in this subclass.

In order to obtain a more complete understanding of the biological aspects of the Erythropeltidaceae it is evident that there are a number of additional areas which must be studied. However, until the formulation of a culture technique which allows the laboratory observation of the complete life cycle of a particular representative, this goal will be difficult to attain. This is particularly applicable in regard to sexual reproduction. It is also evident that electron microscope studies will be a prerequisite to proper documentation of such phenomena. A dependable culture system would also aid in the application of current electron microscopic cytochemical and autoradiographic techniques in an effort to conclusively describe the subcellular mechanisms of sporogenesis.

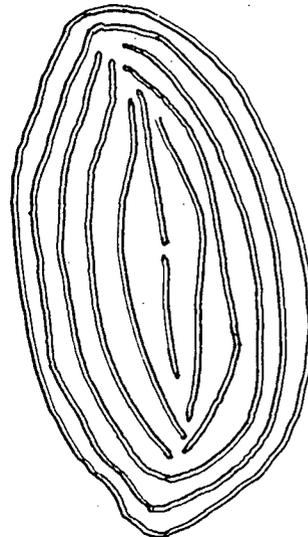
PLATE XL

A bilateral scheme on the evolution of growth types in the Bangiophycidae primarily based on the difference in chloroplast structure shown in the lower portion of the plate.

XL



TYPE I



TYPE II

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