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Taxonomic Implications of Sporanglial Ultrastructure Within the Subfamily Melobesioideae Corallinales, Rhodophyta)

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TAXONOMIC IMPLICATIONS OF SPORANGIAL ULTRASTRUCTURE WITHIN THE SUBFAMILY MELOBESIOIDEAE (CORALLINALES, RHODOPHYTA)

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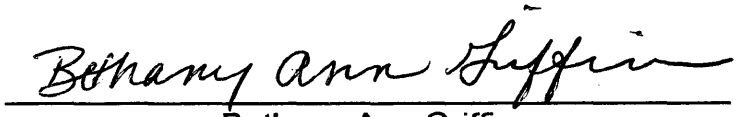

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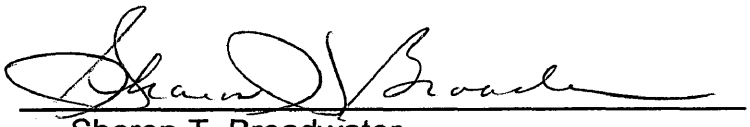

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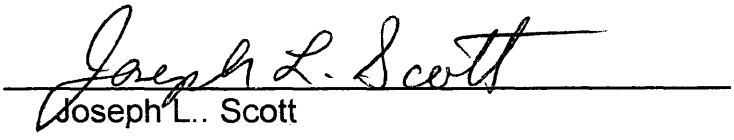

Joseph L.. Scott

Martha A. Case

DEDICATION

To Jon, for giving new meaning to the word supportive.

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ABSTRACT

This investigation examined sporangial development in two genera of coralline red algae, *Lithothamnium* and *Mesophyllum*, to determine ultrastructural features useful for systematic analysis and to clarify the confusing state of coralline classification at the subfamily level. With this study, a total of four genera and six species have been investigated in the non-geniculate subfamily Melobesioideae. All melobesioidean genera lacked nuclear associated electron-dense material (EDM), the presence of which is typical of all other subfamilies having cells that undergo cell fusion. This lack may reflect other evidence that indicates that the Melobesioideae are more primitive and may have separated prior to EDM establishment. Organelle nuclear associations, vesicle types, and pattern of starch organization were present but variable and unlikely to be useful characters at the subfamily level. This is the first electron microscopic study of paraphyses, a group of interspersed, vegetative cells within conceptacles. Apparent lack of calcification was confirmed, as well as, the presence of elongate semi-degenerated cells. However, this study reports the existence of a second, truncated cell form and the presence of a multinucleate condition in some paraphyses cells. In conjunction with supporting molecular data, this study presently favors the taxonomic scheme of Cabioch & Chamberlain over Johansen & Woelkering, with the possibility that more ultrastructural information will warrant the establishment of a new scheme.

TAXONOMIC IMPLICATIONS OF SPORANGIAL ULTRASTRUCTURE WITHIN THE SUBFAMILY MELOBESIOIDEAE (CORALLINALES, RHODOPHYTA)

INTRODUCTION

This ultrastructural investigation of sporogensis in two genera, *Lithothamnium* and *Mesophyllum*, contributes to the body of knowledge to be utilized in determining a more phylogenetic system of classifaction at the subfamily level for the coralline red algae (Corallinales, Rhodophyta). Specific objectives of this research include establishment of a consistent sporangial development pattern for both the Melobesioideae and the Corallinaceae, characterization of paraphyses structure, and determination of the taxonomic usefulness of sporongenesis features to better understand relationships within the Corallinaceae.

Whereas taxonomy is the classification of organisms into groups, systematics is a broader discipline whose main goal is to derive correct phylogenetic relationships through the use of information such as taxonomy. Central principles that define systematic investigation include identification, description, and naming of species, with emphasis on synthesizing biological information into a classifaction system that reflects the phylogenetic history of organisms. Systematics is a basic component of any study of biodiversity and is especially important in the fields of ecology, ethnology, and evolution (Savage 1995).

Scientific investigations that have been important to classification within the phylum Rhodophyta include gross vegetative and reproductive morphology and life history features (Dixon 1973, Gabrielson & Garbary 1986), DNA sequence analysis (Bailey & Chapman 1996, Freshwater *et al.* 1994, Ragan *et al.*

1994), cladistic analysis (Gabrielson *et al.* 1985, Gabrielson & Garbary 1987) and ultrastructure (Pueschel & Cole 1982, Pueschel 1990, Scott & Broadwater 1990). Ultrastructural studies have revealed a new perspective on possible relationships among the various taxonomic levels of red algae while also contributing to a more accurate definition of the phylum Rhodophyta as a whole (Duckett & Peel 1978).

Some ultrastructural aspects that have proven to be important for the classifaction of taxa within the red algae are Golgi associations (Scott 1984), pit plug structure (Pueschel 1987, 1989, Pueschel & Cole 1982), and cell division (Scott & Broadwater 1990). It is the continued goal of this laboratory to use ultrastructural information to elucidate phlyogenetic relationships within the corralline red algae (Wilson 1993, Dearstyne 1994, Hanke 1994, Mansfield 1994, Agee 1995, Agee & Broadwater 1995, Karnas 1995, Lapointe 1995, Griffin & Broadwater 1996, Mays *et al.* 1996, Broadwater & Lapointe 1997).

Rhodophyta is an enigmatic group of organisms whose phylogenetic relationship to other extant organisms is not well understood. Red algae are characterized as eukaryotic, photosynthetic, nonflagellated organisms. They were once considered to be among the most primitive eukaryotes (Garbary & Gabrielson 1990), but recent DNA sequence and ultrastructural evidence indicates that red algae may have diverged in the same time period as higher plants and animals (Bhattacharya *et al.* 1990, Scott & Broadwater 1990). Since phylogenetic relationships are depicted by hierarchical clustering of species in relationship to common ancestor (Savage 1995), the phylogenetic position of Rhodophyta cannot be adequately defined due to both an undetermined divergence point from other organisms and an absence of extant relatives

(Broadwater *et al.* 1992). Even so, the field of red algal systematics has just undergone a dynamic period of change with the likely possibility that the past changes will be viewed as minor compared to those likely to occur in the future (Murray & Dixon 1992).

Red algae are primarily marine, occurring in all biogeographic zones at both benthic and littoral habitats (Gabrielson *et al.* 1991). The number of species is estimated to be between 2,500 to 6,000 (Woelkerling 1990) with a broad range of morphological features encompassing unicells through large pseudoparenchymatous thalli (Gabrielson & Garbary 1986). Nevertheless, Rhodophyta is considered to be monophyletic (Murray & Dixon 1992).

This assumption of monophyly is based on a number of characteristics including lack of a flagellar apparatus in any stage of the life history, the presence of chloroplasts comprised of single, unstacked thylakoids with attached phycobilisomes containing three distinctive phycobilin pigments (allophycocyanin, phycocyanin,and phycoerythrin) and the presence of Floridean starch, a food reserve stored in granules outside the plastid (Gabrielson *et al.* 1985). Other characteristics present in most but not all red algae include the presence of an unusual triphasic life history and unique intercellular connection referred to as a pit connection.

Primary pit connections are produced when a proteinaceous "plug" is deposited within an aperture remaining after incomplete cytokinesis. This 'pit plug complex' of plug and opening is commonly referred to as a pit connection, although there is no cytoplasmic continuity between cells. The structure of pit plugs varies with the presence/absence of cap membranes and the number and

shape of plug caps; this variability has proven to be taxonomically important in the ordinal classification of red algae (Pueschel 1990).

Most multicellular red algae have triphasic life histories composed of haploid, dioecious gametophytes, a diploid carposporophyte, and a diploid tetrasporophyte (Diagram 3). The gametophyte and tetrasporophyte phases exist as independent thalli (which may or may not be morphologically similar); the carposporophyte phase is comparatively smaller and is epiphytic on the female gametophyte (Gabrielson *et al.* 1985).

During sexual reproduction nonflagellated male gametes called spermatia are passively carried by water currents to the female thallus, where they fuse with the trichogyne, an extension of the female gamete called the carpogonium. Fertilization occurs when one of two spermatial nuclei migrates down the trichogyne and fuses with the carpogonial nucleus to produce a diploid zygote. The zygote remains on the female gametophyte and develops into a microscopic carposporophyte consisting of a mass of generative tissue called gonimoblast filaments. Gonimoblast filaments give rise to carposporangia, that in turn, produce carpospores. Released carpospores germinate to produce the diploid tetrasporophyte (Woelkering 1988).

Features of sporangia and spores may vary and thus are potentially useful characteristics for systematic studies (Guiry 1990). Examples of this variation include (but are not limited by) organelle and plastid associations with the nucleus, position of pre-meiotic nucleus, and pattern of cell division in tetrasporangia (cruciate, tetrahedral, or zonate), nature and mechanism of starch deposition, and the mechanism and timing of cytokinesis (Johanson 1981).

Currently, a single class of red algae, Rhodophyceae, comprised of 17 orders is recognized (Garbary & Gabrielson 1990). Members of the Corallinales, the red algal order of interest in this study, are both diverse and copious worldwide. They constitute an important part of benthic communities (Steneck 1986) and contribute to coral reef building by providing structural integrity either through acting in a cementing capacity or by absorbing wave energy (Littler & Littler 1995).

Coralline red algal classification has undergone an extensive history of change starting in the seventeenth century with Linnaeus' determination that corallines were compound coral animals referred to as 'zoophytes' (Gabrielson *et al.* 1991). It was not until the mid-eighteenth century that corallines were considered to be red algae. At the beginning of the twentieth century, coralline red algae were placed in the family Corallinaceae within the order Cryptonemiales (in Gabrielson *et al.* 1991). It is only recently that Silva and Johansen (1986) transferred the family Corallinaceae to its own order, the Corallinales.

Corallinales are distingushed from the other red algae by the presence of calcium carbonate deposited as calcite in cell walls which gives the thallus its structural hardness. In addition to calcification, corallines (with exceptions defined below for the Sporolithaceae) are differentiated from other red algae on the basis of the following characteristics: simultaneously divided zonate tetrasporangia, reproductive cells in roofed conceptacles, an epithallium (an epidermis-like covering over most calcified surfaces) (Johansen 1981), and pit connections in which pit plugs lack a cap membrane but have two cap layers, with the outer one being enlarged and dome shaped (Pueschel 1990).

Presently, Corallinales, a presumed monophyletic taxon, consists of two families, Sporolithaceae and Corallinaceae. Sporolithaceae contains only two genera which are characterized by cruciate tetrasporangia, presence of both secondary pit plugs and cell fusions, and absence of tetrasporangial conceptacles. The second family, Corallinaceae, consists of thirty-eight genera characterized by simultaneous zonate division in tetrasporangia and presence of conceptacles (Verheij 1993). Although the ordinal and family status of this group seems secure, subfamily classification within the Corallinaceae is less certain. A number of characteristics are used to distinguish among the subfamilies of the Corallinaceae; two of the most important are gross morphology and the type of intercellular connection present.

Morphologically, coralline red algae are described either as geniculate or non-geniculate. Geniculate coralline algae have uncalcified regions called genicula that alternate with calcified regions called intergenicula; this pattern enables the upright thallus of the coralline to withstand the buffering effects of waves because of its flexibility. Conversely, non-geniculate corallines are totally calcified, generally forming a flat crust (Johansen 1976) and are often, therefore, often referred to as "crustose" forms .

Although all coralline algae have primary pit connections between kindred cells, two other types of intercellular connections may also be present, secondary pit connections (Diagram 1) or cell fusions (Diagram 2). A secondary pit connection occurs between two cells that have not shared the same mitotic event. In most red algae, a secondary pit connection is formed when one cell cuts off a small cell called a conjunctor cell which fuses with an adjoining, non-

kindred cell. The two non-kindred cells then share a secondary pit-connection, with the receiving cell acquiring a nucleus (Goff & Coleman 1984). However, Corallinales (as well as Hildenbrandiales) have a secondary pit-connection in which two non-kindred cells fuse directly without a conjunctor cell. The pit plug then forms without nuclear exchange having taken place, leaving both cells uninucleate (Cabioch 1971, Pueschel 1988). Another process by which a cellular connection can be made is a cell fusion which result from the cytoplasmic union of two or more vegetative cells to form a large multinucleated cell (Pueschel 1990).

Two competing taxonomic schemes are presently in use and differ in respect to primary character treatment. The most commonly used scheme, proposed by Johansen (1981) and Woelkerling (1988), first divides corallines into two major subdivisions according to gross morphology. This scheme (Table 1) emphasizes the non-geniculate versus geniculate nature of thalli and divides all members of the Corallinaceae into five non-geniculate and three geniculate subfamilies. The geniculate corallines are further classified on the basis of three different types of genicula determined according to their morphology and development (Johansen 1981). Since crustose corallines lack this geniculate patterning, they are further divided based on the type of intercellular connections and type of tetrasporangial conceptacles (uniporate or multiporate).

The opposing scheme, proposed by Cabioch (1971) and Chamberlain (1978) does not use geniculate/non-geniculate characters; instead, primary importance is given to the type of cellular connections present (Table 2), which sometimes results in subfamilies containing both geniculate and non-geniculate

genera. This scheme recognizes six subfamilies within the family Corallinaceae; five subfamilies possess either secondary pit connections or cell fusions, while one, Choreonematoideae, lacks both types of intercellular connections. Both schemes emphasize conceptacle morphology (uniporate/multiporate) at the same character treatment level.

Although the taxonomic scheme of Johansen (1981) and Woelkerling (1988) is used most extensively at the present time, preliminary studies of sporogenesis patterns (Wilson 1993, Dearstyne 1994, Hanke 1994, Mansfield 1994, Agee 1995, Agee & Broadwater 1995, Karnas 1995, Lapointe 1995, Griffin & Broadwater 1996, Mays *et al.* 1996, Broadwater & Lapointe 1997) are more consistant with the second pattern by Cabioch (1971) and Chamberlain (1978) or suggest that a new scheme may be required. Studies using 18s ribosomal gene sequence analysis by former William and Mary graduate student Craig Bailey (1996) also appear to support these conclusions.

Melobesioideae, the subfamily of interest in this study, consists entirely of non-geneculate corallines and is treated identically in both taxonomic schemes due to the unique presence of multiporate tetrasporangial/bisporangial conceptacles. Besides multiporate tetrasporangial/bisporangial conceptacles, this subfamily is characterized by the presence of cell fusions and absence of secondary pit connections, apical pore plugs (Woelkerling 1988) and presence of uncalcified cells collectively called the paraphysis. Paraphysis (or cavity cells) are cells of interspersed filaments residing within conceptacles (Johansen 1981, Townsend 1981, Woelkerling 1988).

There are three main objectives to this investigation: 1) to establish if the Melobesioideae has a consistent sporagensis pattern and if this pattern remians consistent when compared to other Corallinaceae genera 2) to characterize paraphyses structure, and 3) to determine if features of sporogenesis could be useful characteristics to clarify the classifaction within the family Corallinaceae.

A previous William and Mary honors thesis study investigated sporogenesis in one member of the subfamily Melobesiodeae, *Melobesis mediocris* (Agee 1995). The present study of sporogenesis adds two genera, *Lithothamnium* and *Mesophyllum*, to determine the degree of conformity in sporogenesis patterns within the subfamily. Agee (1995) determined that the developmental stages of tetrasporogenesis and carposporogenesis were similar. Her evaluation of sporogenesis patterns revealed a lack of perinuclear electron dense material (EDM), the presence of mitochondrial and plastid perinuclear associations, a progressive developmental sequence of Golgi vesicles, and association of endoplasmic reticulum during starch production (Agee 1995).

Further work by Agee & Broadwater (1995) on *Melobesis marginata* confirmed that these two species are nearly identical at the ultrastructural level. An ultrastuctural survey of sporogenesis developmental stages in *Lithothamnium* and *Mesophyllum* will further assess if the Melobiosiodeae possess any shared patterns that could be used for systematic analysis of all subfamilies. Useful taxonomic characteristics will be determined by comparison of sporangial features among examined species of the Corallinaceae.

Furthermore, an ultrastructural examination of the paraphyses structure of *Lithothamnium* and *Mesophyllum* will be undertaken. Cells of the paraphyses

are presumed to undergo decalcification and become semi-degenerate structures (Johansen 1981, Townsend 1981, Woelkerling 1988), though very little information has been published on this structure. This report will constitute the first electron-micrograph study of these structures.

Sporangial development has been investigated for a number of Corallinaceae members. Various geniculate genera (Peel *et al.* 1973, Duckett & Peel 1978, Vesk & Borowitzka 1984, Wilson 1993, Dearstyne 1994, Hanke 1994, Mansfield 1994, Karnas 1995, Mays *et al.* 1996), a parasite (Lapointe 1995), and one non-geniculate genus (Agee 1995, Agee & Broadwater 1996) have been examined in detail.

EDM was initially described for *Corallina officinalis* (Peel *et al.* 1973) and *Haliptilon cuvierii* (Vesk & Borowitzka 1984) and is presumed to be a region of ribonucleic acid (Vesk & Borowitzka 1984). In this laboratory, the presence of EDM has been shown to correlate with the presence of cell fusions with two distinguishable perinuclear variations of EDM characterized (Wilson 1993). To date, the only exception to the pattern is the genus *Melobesia*, the only representative investigated to date within the subfamily Melobesiodeae (cell fusions). *Lithothamnium* and *Mesophyllum* will be examined to determine if they possess any nuclear associations and whether EDM is present/absent around the nucleus. At present, nuclear associations such as electron-dense material are presently the most promising ultrastructural characteristics for understanding taxonomic relationships at the subfamily level of the Corallinaceae (Wilson 1993, Agee 1995, Agee & Broadwater 1995, Karnas 1995, Griffin & Broadwater 1996, Mays & Scott 1996).

MATERIALS AND METHODS

Transmission Electron Microscopy

Lithothaminum phymatodeum and *Mesophyllum lamellatum* were collected at Bodega Head, California on Novemeber 11 and November 30,1994, respectively. Specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer with 2.5% EDTA and 0.25 M sucrose at pH 6.8 for 30 min at room temperature, followed by an additional 2 hrs of fixation at 4 °C. Specimens were rinsed three times in the above buffer for 10 mins, post-fixed in 1% osmium tetroxide in EDTA-sucrose 0.1 M buffer for 2 hr, and then dehydrated in a gradual acetone series. When dehydration reached 70% acetone, specimens were placed in a 70% methanol-2% uranyl acetate solution overnight at 4 °C and then placed in 100% acetone. The specimens were gradually infiltrated with EMBED 812 epoxy embedding medium, followed by embedding in pure resin at 70 °C for three days.

Lithothaminum glaciale was recieved from Curt Pueschel. Specimens were collected on July 23, 1989 at Land's End, Maine and were fixed for 2 hrs in 5% glutaraldehyde in 0.1 M cacodylate with 0.2 M sucrose. Specimens were rinsed in 0.2 M sucrose buffer with 5% EDTA for up to a week and then placed in a 2% osmium tetroxide overnight. Dehydaration occured through a gradual acetone series of 10 min intervals and rinsed three times in 100% acetone. Specimens were infiltrated and embedded in Spurr's resin, than soaked in 2% uranyl acetate for 1.5 hrs.

All embedded specimens were trimmed and sectioned on an RMC MT 6000X ultramicrotome. Tissue sections were stained for 1 min in SATO's lead hydroxide and transfered to formvar coated one-hole grids. Grids were photographed using T-max 100 film on a Zeiss EM 109 electron microscope.

Scanning Electron Microscopy

Air-dried specimens of *Lithothamnium phymatodeum* and *Mesophyllum lamellatum* were dehydrated with ethanol and crictically point dried using the samdri-PVT-3B. Dried specimens were mounted using conductive graphite adhesive on aluminum studs and coated with 20nm of gold-palladium in a Hummer VII sputter-coater. Specimens were photographed using T-Max 100 film on an AMRAY 1810 scanning electron microscope.

Light Microscopy

Dried specimens of *Lithothamnium phymatodeum* and *Mesophyllum lamellatum* were photographed using Extachrome 100 film on the WILD Photomarkos KOP 400.

RESULTS

General Thallus Construction

Lithothamnium phymatodeum grows on rough strata such as shell, stone, or other algae. It forms a pink, brittle, continuous crust that has irregular protuberances and crowded patches of conceptacles (Figs.1, 3-6). The thallus, which is approximately 2 mm thick (Figs. 3, 5), is monomerous and composed of a core of filaments whose distal portions curve upwards towards the crust's surface (Diagram 4). Each protuberance consists of a middle coaxial central core (medulla) of filaments with peripheral filaments that arch toward the surface (cortex) (Figs. 3, 4).

Mesophyllum lamellatum grows normally on geniculate coralline algae (Fig. 2). The monomerous thallus (Diagram 4) consists of pale pink crusts overlapping in a shingle-like construction (Figs. 2, 7). The crusts are approximately 1 mm in thickness (Figs. 8, 9).

General Conceptacle Construction

Raised bisporangial conceptacles of *Lithothamnium* occur in dense groupings that cover most of the entire thallus except on protuberances (Figs. 1, 3-6); the surface of *Mesophyllum's* crustose thallus appears much smoother since there are no protuberances (Figs. 2, 7-9) and tetrasporangial conceptacles are not significantly raised above the plane of the surrounding thallus (Figs. 2, 7-9). Bisporangia observed in two *Lithothamnium* species

(Figs. 6, 10, 29, 31) and tetrasporangia found in *Mesophyllum* (Figs. 9, 30) possess pore plugs and are housed in multiporate conceptacles. In both genera, conceptacle roofs are two to several cells thick (Fig. 16). *Lithothamnium's* bisporangial conceptacles are approximately 210 μm in height and 380 μm in width (Figs. 5,10,16), while *Mesophyllum's* tetrasporangial conceptacles average 425 μm in height and 335 μm in width (Figs. 8, 9, 18).

Paraphyses Structure

There are no essential differences in paraphyses structure between *Lithothamnium* and *Mesophyllum* so the following description will make no distinction with regard to genus (Figs. 10-24).

Paraphyses in mature conceptacles encapsulate conceptacle contents in a continuous seamless shell (Fig. 11). As observed by SEM, no calcification is detectable in any cells of the paraphyses with the possible exception of cells adjacent to the conceptacle (Figs. 11-13). Calcium carbonate walls with intact cells are found on the exterior of paraphyses (Figs. 12, 13). These small, spherical/cuboidal cells average 4.3 μm in diameter, but their origin could not be determined. Since specimens were dried causing paraphyses to pull away from the conceptacle, the cells could be the outermost cells of the paraphyses or the innermost cells of the conceptacle (Figs. 11-13).

Uncalcifed cells of paraphyses line the inside of the conceptacle chamber (Figs. 14-17) producing a smooth multilayered lining (Fig. 14). Remnants of pit connections are evident in cells of paraphyses that line the bottom of the inner conceptacle chamber floor (Figs. 15, 17). One or two apparently uncalcified

sheaths of paraphyses cells encase each sporangium and extend from the bottom to the inside top of the conceptacle chamber (Figs. 16, 18-20).

Paraphyses cells that surround bisporangia are elongate with a recorded length of up to 59 μm and width of 5 μm (Figs. 21, 22, 24), while those that line the conceptacle chamber are irregular and more truncated in appearance with an average length of 27 μm and width of 25 μm (Figs. 23, 33). In either case, the majority of cells contain little cytoplasm with minimal numbers of organelles; cell interiors are primarily vacuolate with most organelles located at the periphery or surrounding the nucleus (Figs. 21-24). Unlike other cells of the thallus, those of the *Lithothamnium*'s paraphyses are multinucleate with an average of two to four nuclei per thin section (Figs. 22, 23), although in one case seven nuclei were present (Fig. 24).

Sporangial Development

Though both bisporangia and tetrasporangia can be produced by the species in this investigation, only bisporangia were seen in *Lithothamnium* and only tetrasporangia in *Mesophyllum*. At maturity, bisporangia measure up to 160 μm in height and 95 μm in width (Fig. 27), whereas tetrasporangia measure up to 155 μm in height and 60 μm in width (Fig. 28). In both genera, sporangia are found scattered throughout the conceptacle chamber floor.

Spore development is asynchronous so that many developmental stages will be observed within a single conceptacle (Figs. 25, 26). Each sporangium is affixed to the conceptacle by a pore plug that attaches the spore to the conceptacle roof (Figs. 29-31) and a stalk cell that connects it to the bottom

(Figs. 33-36). Smooth endoplasmic reticulum (SER) can be observed clumped at the apical end of a bisporangium apparently producing the contents incorporated into the established pore plug (Figs. 31,32). SER is also present in large quantities near the pit-connections between the bisporangium and stalk cell (Figs. 33-36). SER forms a complete channel within the middle of the stalk cell prior to meiosis (Figs. 33, 34). After meiosis, when the spore wall develops, a wall ring is evident (Figs. 35-36) which surrounds a typical pit connection between the bisporangium and stalk cell (Fig. 36).

Bisporogenesis and Tetrasporogenesis Developmental Stages

The developmental stages of sporogenesis in this investigation follow the designation proposed by Scott & Dixon (1973) and Vesk & Borowitzka (1984) in which Stage 1 refers to pre-meiotic sporangia, Stage 2 to meiotic sporangia, Stage 3 to post-meiotic sporangia prior to full cleavage, and Stage 4 to completely cleaved mature sporangia. Stage 3 is further divided into three substages (A,B,C) on the basis of occurrence and distribution of plastids and vesicles. Since many development features of sporogenesis correspond across species, sporogenesis development of *Lithothamnium phymatodeum* will be described in detail but the development of *Mesophyllum lamellatum* will be addressed only when there are differences. Neither Stage 2 nor Stage 4 sporangia were observed in any species examined in this study.

Stage 1: Pre-meiotic Sporangia

Bisporangia of *Lithothamnium*

Pre-meiotic bisporangia are highly vacuolate and elongate, measuring approximately 8 μm in width and 60 μm in length (Fig. 37). A single, spherical nucleus with smooth contours is located in the mid region of the sporangium (Fig. 37). No organelles or other material are closely associated with the nucleus (Fig. 38). Few organelles are present; those that are, are located in regions near the nucleus or around the periphery of the bisporangium (Figs. 37, 38). Chloroplasts are immature with numerous genophores, 2 to 7 linear thylakoids, and a peripheral encircling thylakoid (Fig. 38). Although sporadic, Golgi bodies contain up to 9 straight or cup-shaped cisternae which are always associated with mitochondria (Fig. 41). Vesicles are not associated with the Golgi nor found in the cytoplasm (Figs. 38, 41). A single cisternum of rough endoplasmic reticulum (RER) is found intermittently along the periphery of the sporangium (Fig. 41).

Tetrasporangia of *Mesophyllum*

Pre-meiotic tetrasporangia (Figs. 39, 40) follow the same Stage 1 pattern as above.

Early Stage 3A: Post-meiotic Sporangia

Bisporangia of *Lithothamnium*

Aftercompletion of meiosis, early Stage 3A bisporangia remain highly vacuolate with little evidence of growth (Fig. 42). Daughter nuclei, which are located in the center of the nascent spores, remain devoid of organelle

associations (Fig. 43). Starch production begins and grains are associated both with vacuoles and ER (Figs. 42-45). Starch is either appressed on both sides by ER (Fig. 45) or in between a vacuole and ER (Figs. 44, 45). The numbers of mitochondria and chloroplasts increase (Figs. 42, 44). Most chloroplasts remain at the periphery of the bisporangium in proximity to tracks of ER (Fig. 44). Chloroplast profiles are somewhat more immature with 2-4 non-continous thylakoids and fewer genophores (Fig. 44). Golgi are cup-shaped and more numerous (Figs. 43, 44), but vesicles are not evident in this stage (Figs. 43, 44). Zonate cleavage furrows are present (Fig. 42).

Tetrasporangia of *Mesophyllum*

Degree of vacuolation, type of vesicle production, and starch deposition differ in this genus during early Stage 3A. Vacuoles greatly decrease both in number and volume, while starch grains are not present (Figs. 46-51). Osmiophillic vesicles, produced by Golgi (Figs. 49-51), appear for the first time as do two types of cored vesicles (Figs. 46-50). All are also abundant in the cytoplasm. One type, a large-cored vesicle, has an average diameter of 1.2 μm and a center whose diameter is almost as large as that of its vesicle, with an average diameter of 1.1 μm (Figs. 47, 48). The other dark-cored vesicle (average diameter of 1.4 μm) , has a very electron dense core (average diameter of 0 .6 μm) surrounded by diffuse contents (Figs. 46, 47, 49, 50). In some tetrasporangia, dark-cored vesicles appear donut-like (Fig. 49). Concentric layers of membranous material transiently appear during this stage (Figs. 50, 51). The plasmalemma is irregular and a thick wall surrounds the tetrasporangium (Fig. 46).

Late Stage 3A: Post-meiotic Sporangia

Bisporangia of *Lithothamnium*

By late stage 3A, bisporangia increase in width, averaging 20 μm (Fig. 52). Each nucleus is associated with numerous chloroplasts and mitochondria (Fig. 53). Starch production continues with deposition between parallel cisternae of ER and between ER and vacuolar membranes (Figs. 52-54), although vacuole numbers are significantly reduced (Figs. 52, 53). Proplastids increase significantly in number, but remain immature (Figs. 53, 54). Increased numbers of osmiophillic vesicles are located in clumps (Figs. 52, 56); only a few lipid globules (not shown) and irregular granular vesicles are present (Fig. 56). Some smooth-textured vesicles are also produced by the Golgi at this time (Fig. 55), but no type of dark-cored vesicles can be observed.

Tetrasporangia of *Mesophyllum*

Late Stage 3A in this genus differs from the above by an absence of any nucleus/chloroplast/mitochondria association, near absence of vacuoles, and type of vesicle production. No associations between the nucleus and other organelles exist in this stage (Figs. 57, 58). Chloroplasts increase in number and appear somewhat better developed (Figs. 58, 60). Vacuoles are few and isolated, while starch is located parallel to ER cisternae (not shown). No types of cored vesicles or smooth-textured vesicles are present in the cytoplasm (Figs. 57, 58, 60) Two granular vesicles are present, one regular form with smooth contours (Fig. 61), the other irregular with variable contours (Figs. 59, 60).

Stage 3B: Post-meiotic Bisporangia

Bisporangia of *Lithothamnium*

In Stage 3B, the diameter of bisporangia (Fig. 62) is doubled compared to that of initial post-meiotic bisporangia (early Stage 1A). The nuclear association with chloroplasts and mitochondria persists (Fig. 63). Vacuoles essentially disappear but parallel stacks of starch grains between ER cisternae continue to increase in number (Figs. 62, 63, 66). Starch grains are lenticulate or ovate in shape (Figs. 62, 63, 66). Chloroplasts have many fewer genophores but possess 7 to 9 fully-formed thylakoids (Figs. 63, 64). Chloroplast division is prevalent and evidenced by an increased number of profiles and the presence of an osmiophillic structure between some chloroplast lobes (Fig. 64). Osmiophillic vesicles increase greatly in number forming clumps (Figs. 62-65), while lipid globules are more abundant (Figs. 65). No granular or smooth-textured vesicles are present.

Tetrasporangia of *Mesophyllum*

Stage 3B post-meiotic tetrasporangia in this genus (Figs. 67-71) deviate from the former by the presence of starch in association with the nucleus (Fig. 68) and by the difference of electron opacity of osmiophllic vesicles (Figs. 69-71), although the latter may be an artifact of staining. Simultaneous zonate cleavage is arrested (Fig. 67).

Stage 3C: Post-meiotic Sporangia

Bisporangia of *Lithothamnium*

In Stage 3C, bisporangia have reached their maximum volume, with a length of approximately 195 μm and a width of 65 μm (Fig. 72). The nuclear association with chloroplasts and mitochondria remains, but the nucleus becomes irregularly shaped (Figs. 72, 73). Vacuoles are no longer evident (Figs. 72-76) and starch grains acquire a somewhat crenulate appearance (Figs. 74, 75). Chloroplasts, starch, osmiophillic vesicles, and lipid globules become dispersed (Figs. 72, 74-76). Chloroplasts are mature with many thylakoids and few genophores (Figs. 73-75). Small electron-translucent vesicles are present but dispersed as well (Figs. 74, 76). SER parallels the cleavage furrows (Figs. 74, 76).

Tetrasporangia of *Mesophyllum*

The only difference between Stage 3C tetrasporangia (Figs. 77-79) from Stage 3C bisporangia is that no nuclear association is evident (Fig. 78). Even the transient starch association observed in the former stage is absent.

DISCUSSION

Paraphyses Structure

At maturity, all melobesiodean genera have decalcified conceptacle filaments collectively called paraphysis (or cavity cells) interspersed among developing sporangia (Townsend 1981, Johansen 1981). Paraphyses are thought to arise from calcified interspersed conceptacle filaments that subsequently decalcify and undergo elongation (Johansen 1981). As the growing sporangia crowd the paraphyses, most cells of the paraphyses become either semi-degenerate or disappear entirely. The presence of these uncalcified vegetative filaments amongst developing sporangia is believed to be a less advanced condition than those taxa that lack them entirely (Johansen 1988). This is the first electron microscopy study of paraphyses structure in mature conceptacles. No differences in ultrastructure were observed between *Lithothamnium* and *Mesophyllum* except in regards to the presence of multinucleate condition in some paraphysis cells of *Lithothamium*.

Determining the structural relationships of uncalcified cells through scanning electron microscopy is difficult (Garbary 1978). In this study, it was often difficult to determine cell boundaries, but this examination supports earlier light microscopic studies indicating a loss of calcification in these structures (Johansen 1981) with the possible exception of the cell layer adjacent to the conceptacle. Cell walls with calcium carbonate were observed in this study but the origin of these cells could not be explicitly determined. They were either the

outermost cells of the paraphyses or they were the innermost cells of the conceptacle which were pulled from the inner conceptacle wall as the paraphyses material shrank during drying.

In both scanning and transmission electron microscopy, paraphyses sheaths around developing sporangia appear to be one to two layer(s) thick and very elongate. In contrast, cells that line the conceptacle chamber seem to be more rounded and truncated in appearance. Though marked elongation of paraphyses cells had been observed previously (Adey & Johansen 1972, Johansen 1981, Townsend 1981), the other truncated form was not addressed in previous investigations.

Multinucleated cells were observed in two *Lithothamnium* species, *L. phymatodeum* and *L. glaciale*. though multinuclearity is common in red algae (Pueschel 1990), members of the Corallinaceae usually acquire multinucleate vegetative cells only through cellular fusions (Johansen 1981). Until this investigation, the only noted exceptions were some multinucleate vegetative cells in two coralline parasites, *Austrolithon intumescens* (Harvey & Woelkerling 1995) and *Choreonema thuretii* (Lapointe 1995, Broadwater & Lapointe 1997). Some paraphyses cells of *Lithothamnium* contain two to four nuclei as observed in one plane of sectioning, and in one case seven. It is unlikely that this multinucleate condition is the result of cell fusion, based on the number of nuclei present in each cell and the proximity of multinucleate cells to each other. The function of this condition, however, is unknown.

Sporogenesis

Determining sporangial developmental patterns could be beneficial in constructing phylogenetic relationships (Guiry 1990). Sporangial development consists of cell events that are indicative of a form/function relationship. Developmental events include sporangial elongation, varied production and increased accumulation of cellular reserves, and distributional changes of vesicles and organelles (Guiry 1990). Results of this investigation indicate that for some aspects of sporogenesis a consistent development pattern exists for Melobesioideae and continues to be upheld within all examined genera of the Corallinaceae.

General Bi/Tetrasporangial Developmental Pattern For Corallinaceae

During development, sporangia (carpospores unadressed) follow a general pattern to prepare for division, maturity, and release (Table 4). Pre-meiotic/mitotic sporangia (Stage 1) are very vacuolate and contain a limited number of organelles and no vesicles. Chloroplasts are mature in the very earliest part of Stage 1. Since meiosis/mitosis (Stage 2) occurs quickly, not enough data has been collected to determine any definitive pattern for this stage. Post-meiotic/mitotic sporangia (Stage 3) can be divided into three substages. In Stage 3A, vacuolation diminishes, osmiophillic vesicles are produced, and starch production commences. Chloroplasts become proplastid-like with many genophores and few thylakoids. Simultaneous zonate cleavage is initiated and ER is found at the periphery of the sporangium. In Stage 3B, vacuoles disappear, osmiophillic vesicle production continues forming clumps of vesicles,

and starch production increases. New starch is organized in parallel stacks with ER cisternae. Chloroplasts remain immature but increase greatly in number. Cleavage is arrested. Stage 3C is marked by dispersion of osmiophillic vesicles, organelles, and starch. Chloroplasts mature during this stage, have fewer genophores and contain an increased number of thylakoids per plastid. Mature spores (Stage 4) have not been observed ultrastructurally in this investigation

When interpreting this general pattern, the form/function relationship suggested by Guiry (1990) appears to be correct. Developing sporangia undergo a series of drastic changes in order to become mature spores. Nascent sporangia arise from vegetative tissue to become reproductive tissue, change from diploid to haploid, and transform from part of a multicellular thallus to a single cell. In Stage I, sporangia have minimal cellular contents and are preparing for meiosis/mtiosis and in the case of tetrasporangia, the change from diploid to haploid. Once meiosis/mitosis is completed, Stage 3A sporangia elongate and commence high production of organelles and vesicles. Most variation occurs during this stage as the cell turns on new genes and produces new proteins, while increasing its necessary cellular contents in order to prepare developing spores for the transformation from vegetative tissue to reproductive tissue. From Stage 3B until mature sporangia are produced, little variation occurs; during this time, sporangia are growing and organizing and refining their contents in preparation for release. Completion of simultaneous zonate cleavage in Stage 4, completes the change from part of a multinucleate thallus to a single cell.

Bi/Tetrasporangial Development in the Melobesiodeae

A general sporogangial (carpospores exculded) development pattern for the Corallinaceae was established on the basis of certain invariable sporangial features that included cell wall, chloroplasts, vacuoles, starch, and osmiophillic vesicle distribution. Sporangial development in the family Corallinaceae follows this pattern as well. However, there are sporangial features with varying degrees of consistency (Table 5). Specific characteristics, including nuclear associations, Golgi body production and vesicle type, and starch organization, are evaluated below for their taxonomic importance.

Nuclear Associations

Nuclear associations are potentially useful taxonomic characters. The arrangement of various organelles, plastids, and electron dense material (EDM) around the nucleus has been established for the majority of coralline subfamilies. To date, 19 genera of coralline red algae have been investigated for nuclear associations and other details of sporangial development (Table 3) (Peel *et al.* 1973, Vesk and Borowitzka 1984, Wilson 1993, Agee 1995, Agee & Broadwater 1995, Karnas 1995, Lapointe 1995, Griffin & Broadwater 1996, Mays *et al.* 1996).

Various nucleus/organelle arrangements include a mitochondria association established in three genera of the subfamily Corallinoideae, *Bossiella* (Wilson 1993, Karnas 1995), *Calliarthron* (Karnas 1995), and *Chiharaea* (Karnas 1995, Mays *et al.* 1996). A chloroplast-mitochondria association is present in the subfamily Melobesiodeae within both *Melobesia* (Agee 1995, Agee & Broadwater 1995) and in this study of *Lithothamnium*. Another type of nuclear association

consists only plastids and was present in *Amphiroa* (Wilson 1993, Dearstyne 1994, Hanke 1994) in the subfamily Amphiroideae, while a starch only association was noted in both *Titanoderma* (Wilson 1993) of the Lithophylloideae and *Lithothrix* (Wilson 1993, Mansfield 1994) of the Amphiroideae. This study also shows that *Mesophyllum* (Melobesiodeae) has a starch only association. Nuclear associations of organelles or starch have not been found to be consistent within subfamilies indicating that these associations are not a reliable taxonomic character at the subfamily level. However, these associations may be potentially useful characters at the genus level.

Another nuclear association is that of EDM. EDM was first described in *Corallina* tetrasporangia (Peel *et al.* 1973) and later described in both *Jania* (Duckett & Peel 1978) and *Haliptilon* (Vesk & Borowitzka 1984). Investigations in our laboratory initially showed a correlation between the presence of EDM with the presence of cell fusions and the absence of EDM with the presence of secondary pit connections. Both *Amphiroa* (Wilson 1993, Dearstyne 1994, Hanke 1994) and *Lithothrix* (Wilson 1993, Mansfield 1994) of the Amphiroideae and *Titanoderma* (Wilson 1993) in the Lithophylloideae lacked EDM and possessed secondary pit-connections.

In contrast, all members of the subfamily Corallinoideae contain cell fusions and have EDM (Duckett & Peel 1978, Wilson 1993, Karnas 1995, Mays *et al.* 1996). Wilson (1993) determined two patterns of EDM in the Corallinoideae, the *Bossiella* type and *Corallina* type. The *Bossiella* type EDM has been found in *Bossiella* and *Calliarthron* (Wilson 1993), *Arthrocardia*, *Chiharaea*, *Serraticardia*, and *Yamadaea* (Mays *et al.* 1996), whereas the

Corallina type has been found *Jania* (Duckett & Peel 1978), *Haliptilon* (Vesk & Borowitzka 1984). Also, in the subfamily Mastophoroideae (possessing cell fusions), the *Corallina* type EDM was present in *Metamastophora*; however, *Hydrolithon*, another member of this subfamily, lacked EDM altogether (Wilson 1993). Besides the Melobesioideae, discussed below, *Hydrolithon* is the only exception to this correlation and needs to be reinvestigated for this reason and due to the preliminary nature of Wilson's examination (1993). Wilson concluded from her survey of nuclear associations, that all examined members of the Corallinoideae possessed EDM and predicted that coralline algae with cell fusions, no secondary pit connections and uniporate conceptacles would also have EDM. Based on this cursory evidence, the non-geniculate genus *Melobesia* was also predicted to have EDM; however, none was found (Agee 1995, Agee & Broadwater 1995). This investigation determined that two other genera of the Melobesiodeae, *Lithothamnium* and *Mesophyllum* also lack EDM, indicating that coralline algae with multiporate conceptacles apparently do not have EDM. A possible explanation could be that melobesiodean algae, which are considered to be the most primitive clade of corallines (Bailey & Chapman 1996), separated from all other coralline algae possessing cell fusions prior to the establishment of EDM.

Golgi Production and Vesicle Types

Vesicles secreted by Golgi and other intermediate vesicles found in the cytoplasm are the most variable component during sporogenesis in terms of type and stage of appearance. Only osmiophillic vesicles have been found in all

genera of the Corallinaceae examined, including *Lithothamnium* and *Mesophyllum* (Duckett & Peel 1978, Wilson 1993, Dearstyne 1994, Hanke 1994, Mansfield 1994, Agee 1995, Agee & Broadwater 1995, Karnas 1995, Lapointe 1995, Mays *et al.* 1996). Osmiophillic vesicles are derived from the Golgi apparatus, form clumps (probably due to an increase of vesicles around the area of production), and then disperse. After initial production in Stage 3A, they are retained throughout sporangial development. Osmiophillic vesicles are thought to contain an adhesive material for newly discharged spores (Vesk & Borowitzka 1984).

Grainy vesicles are suggested to contain mucilage (Vesk & Borowitzka 1984) and have been reported in some coralline genera (Vesk & Borowitzka 1984, Wilson 1993, Agee 1995, Agee & Broadwater 1995, Karnas 1995). One type, the cored-vesicles are first found throughout the cytoplasm in early Stage 3A and disappear quickly by late Stage 3A. Cored-vesicles are found in three out of four melobesiodean genera investigated, *Melobesia* (Agee 1995, Agee & Broadwater 1995), *Clathromorphum* (Griffin & Broadwater 1996), and *Mesophyllum*, but not in *Lithothamnium*. Types of cored vesicles present include dark-cored, large-cored and donut-like vesicles. Average diameters for both large-cored vesicles and dark-cored vesicles are similar; however, the large cores are approximately double the size of the dark cores, suggesting that the large cores may condense into the smaller dark cores. This argument is further strengthened by the sequential appearance of first the large core and then the dark core vesicles. Rapid disappearance of these vesicles supports the idea that

these vesicles contain mucilage; however, to reach a definitive conclusion about vesicle relationships, more ultrastructural evidence is needed.

At present, no real taxonomic value can be placed on vesicle type at the subfamily level. Since osmiophillic vesicles are present in all documented sporangia, it is a constant feature of sporangial development and has no taxonomic importance. Cored vesicle types are found in few genera and their presence is not consistant within subfamilies, making them a poor taxonomic character. Equally sporadic are smooth-textured vesicles found only in *Melobesia* (Agee 1995, Agee & Broadwater 1995) and *Lithothamnium*.

However, vesicles that are specific to a certain subfamily or alga might be of importance at other taxonomic levels. For instance, all four melobesiodean genera studied, *Melobesia* (Agee 1995, Broadwater & Agee 1995), *Clathromorphum* (Griffin & Broadwater 1996), *Mesophyllum* and *Lithothamnium* have granular vesicles in late stage 3A. Two types of granular vesicles have been documented, a regular (circular) and irregular form. Furthermore, the presence of fibrous vesicles in *Choreonema* (Lapointe 1995) is an example of a vesicle type specific to a particular genus.

Devacuolation and Starch Organization

In contrast to green algae and higher plants, red algal Floridean starch is present in the cytoplasm and often in the vicinity of the RER, nucleus, and other structures unless a pyreniod is present (Pueschel 1990). Two basic types of starch organization have been described. *Lithothrix* (Borowitzka 1978, Mansfield 1994), *Amphiroa* (Dearstyne 1994, Hanke 1994), *Haliptilon*

(Vesk & Borowitzka 1984), *Melobesia* (Agee 1995, Agee & Broadwater 1995), *Choreonema* (Lapointe 1995) and *Mesophyllum* all organize starch between ER cisternae forming linear parallel stacks; whereas, *Bossiella* (Wilson 1993, Karnas 1995) and *Lithothamnium* have starch initially organized around vacuoles. Even so, starch grains are eventually layered between ER and vacuoles and when the vacuoles disappear, starch is left surrounded by ER cisternae.

In either situation, ER cisternae eventually surround the starch in the developing sporangia, which supports the assertion (Borowitza 1978, Vesk & Borowitza 1974) that ER is likely involved in the biochemical formation of Floridean starch. Presence of ER in both cases suggest that vacuoles play a secondary role in starch organization. However, close association of starch with the vacuoles argues against any suggestion of random localization since only starch is seen surrounding the vacuoles, suggesting some kind of unknown functional role for the vacuole in starch deposition.

Taxonomic Implications

Recent 18s ribosomal studies (Bailey & Chapman 1996) showing percent sequence similarity of 23 genera of Corallinaceae (Lithophylloideae unaddressed) indicate the presence of three evolutionary lines, consisting of the Corallinoideae, Melobesioideae, and a group comprised of Amphiroideae, Metagoniolithoideae, and Mastophoroideae (based on only *Spongites yendoi*) (Diagram 5). Melobesioideae was found to be basal to all other coralline clades suggesting it is the least evolutionary advanced group. This investigation upheld the monophyly

of the Melobesioideae which is supported by Johansen (1981) Woelkerling (1988) and Cabioch (1971) Chamberlain (1978) on the basis of multiporate conceptacles.

Molecular evidence (Bailey & Chapman 1996) also indicated that the non-geniculate genus *Spongites* (subfamily Mastophoriodeae) is more closely related to the geniculate subfamilies of Amphiroideae and Metagoniolithoideae than it is to the non-geniculate Melobesiodeae (Bailey & Chapman 1996). Though more genera in the non-geniculate Mastophoriodeae need to be investigated to reach a definitive conclusion, this close relationship between a non-geniculate genus and certain geniculate corallines, as well as, the lack of a close relationship between the Amphiroideae and Corallinoideae suggest that the presence of genicula is not an important character for subfamily classification and that the Johansen (1981) and Woelkerling (1988) scheme is not supported.

On the other hand, molecular evidence also does not fully support the importance of cell fusions versus secondary pit-connections as a primary character in subfamily designation. Two species with cell fusions, *Metagoniolithon* (Metagoniolithoideae) and *Spongites yendoi* (Mastophoroideae) show a closer relationship to the Amphiroideae, which have secondary pit-connections than to the Corallinoideae and Melobesiodeae that have cell fusions. Also, the Lithophylloideae, the only other subfamily that has secondary pit-connections, was unadressed by the molecular data. In order for the scheme by Cabioch (1971) and Chamberlain (1978) to be upheld, the Lithophylloideae will need to show a close relationship with the Amphiroideae.

Ultrastructural studies may uncover important phylogenetic characters that will help to unravel problems resulting from molecular studies. Currently, the presence or absence of EDM is the most promising taxonomic character to help resolve conflicting classification schemes.

EDM has been found in all geniculate genera having cell fusions, and in *Metamastophora* (Mastophoroideae), a non-geniculate genus which also has cell fusions, while the genus Titanoderma (Lithophylloideae) and the Amphiroideae both have secondary pit-conections and no EDM. This evidence correlating EDM with cell fusions compliments 18s ribosomal molecular data (Bailey & Chapman 1996), suggesting that the Johansen (1981) and Woelkerling (1988) scheme may not be phylogenetically credible. However, EDM was not found in *Hydrolithon*, the only other genus of Mastophoroideae investigated. This evidence is the only serious concern with respect to the utility of EDM as a useful character at the subfamily level; consequently, *Hydrolithon* needs to be re-evaluated and other members of the subfamily Mastophoroideae need to be investigated by both molecular and ultrastructure techniques in order to further clarify this situation.

If Cabioch (1971) and Chamberlain (1978) is the more accurate classification scheme, then the presence of EDM might be expected in the Melobesioideae, just like the Corallinoideae, since they both posses cell fusions. However, all four genera of Melobesiodeae studied lack EDM. One possible explanation is that EDM could have evolved after Melobesiodeae diverged from the common ancestor. If this were true, the primary importance of cellular connections in Cabioch (1971) and Chamberlain (1978) 's scheme would still be accurate. However, in light of Bailey & Chapman's (1996) 18s ribosomal studies

showing a close relationship of *Metagoniolithon* (Metagoniolithoideae) and *Spongites yendoi* (Mastophoroideae) with the subfamily Amphiroideae, (thereby linking cell fusions with secondary-pit connections), a new scheme may be warranted.

Future Research

In order to determine if the exterior of the paraphyses contains calcium carbonate, an energy dispersive x-ray analysis should be completed. Also, the unusual multinuclear condition of *Lithothamnium*'s paraphyses should be investigated more thoroughly. Lastly, ultrastructural sporogensis studies of mastophoroidean and lithophylloidean genera, as well as, the Sporolithaceae need to be completed to further resolve phylogenetic relationships.

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Table 1

CORALLINE CLASSIFICATION

according to Johansen (1981) and Woelkerling (1988)

ORDER CORALLINALES

FAMILY SPOROLITHACEAE

Heydrichia
Sporolithon

FAMILY CORALLINACEAE

Amphiroideae
Amphiroa
Lithothrix

Corallinoideae
Alatocladia
Arthrocardia
Bossiella
Calliarthron
Cheilosporum
Chiharaea
Haliptilon
Jania
Marginisporum
Serraticardia
Yamadaea

Metagonioloithoideae
Metagoniolithon

Melobesiodeae
Clathromorphum
Exilicrusta
Kvaleya
Lithothamnium
Mastophopsis
Melobesia
Mesophyllum
Phymatolithon
Synarthrophyton

Lithophylloideae
Ezo
Lithopyllum
Tenarea
Titanoderma

Mastophoroideae
Hydrolithon
Lesureuria
Lithoporella
Mastophora
Metamastophora
Neogoniolithon
Pneophyllum
Spongites

Choreonematoideae
Choreonema

Austrolithoideae
Austrolithon
Boreolithon

* Geniculate Genera are in **BOLD** type.

Table 2

CORALLINE CLASSIFICATION

according to Cabioch (1971) and Chamberlain (1978)

ORDER CORALLINALES

FAMILY SPOROLITHACEAE

Heydrichia
Sporolithon

FAMILY CORALLINACEAE

Melobesiodeae	*Lithophylloideae*	*Corallinoideae*
Clathromorphum	Ezo	Hydrolithon
Exilicrusta	Lithopyllum	Lesureuria
Kvaleya	Tenarea	Lithoporella
Lithothamnium	Titanoderma	Mastophora
Mastophopsis	**Amphiroa**	Metamastophora
Melobesia	**Lithothrix**	Neogoniolithon
Mesophyllum		Pneophyllum
Phymatolithon		Spongites
Synarthrophyton		**Arthrocardia**
		Bossiella
		Calliarthron
		Cheilosporum
		Chiharaea
		Haliptilon
		Jania
		Marginisporum
		Serraticardia
		Yamadaea

Choreonematoideae	*Metagoniolithoideae*	***Austrolithoideae*
Choreonema	**Metagoniolithon**	Austrolithon
		Boreolithon

*Geniculate Genera are in **BOLD** type.

**The subfamily Austrolithoideae comprising two genera has only been recently discovered and characterized by Harvey & Woelherling (1995). According to the critera of the Cabioch and Chamberlain scheme, this subfamily would be recognized on the basis of lacking both secondary pit connections and cell fusions and would be placed as a separate subfamily as indicated.

Table 3

Summary of Characteristics for Corallinaceae Classification

Subfamily	Genus	Genicula	2' Pit Connection	Cell Fusion	Multiporate Conceptacle	EDM	Other Perinuclear Associations
Amphiroideae	Amphiroa	+	+	_	_	_	P, Ch
	Lithothrix	+	+	_	_	_	P, S
Corallinoideae	Bossiella	+	_	+	_	B	P, M
	Calliarthron	+	_	+	_	B	P, M
	Cheilosporum	+	_	+	_	B/C	**NBD**
	Chiharaea	+	_	+	_	B	P, M
	Corallina	+	_	+	_	C	P
	Haliptiion	+	_	+	_	C	P
	Jania	+	_	+	_	C	P
	Serraticardia	+	_	+	_	B	**NBD**
	Yamadaea	+	_	+	_	B	**NBD**
Choreonematoideae	Choreonema	_	_	_	_	_	P
Lithophylloideae	Titanoderma	_	+	_	_	_	S
Mastophoroideae	Hydrolithon	_	_	+	_	_	P,R
	Metamastophora	_	_	+	_	C	P
Melobesioideae	Clathromorphum	_	_	+	+	_	**NBD**
	Mesophyllum	_	_	+	+	_	S
	Lithothamion	_	_	+	+	_	M, Ch
	Melobesia	_	_	+	+	_	M, Ch

Legand: +: present, _: absent, B: Bossiella type EDM, C: Corallina type EDM, Ch: Chloroplast, M: Mitochondria, P: Perinuclear ER, R: Ribosomes, S: Starch, **NBD: Not Been Determined.**

(according to Cabioch 1971, Peel et al. 1973, Borowitzka 1978, Chamberlain 1978, Johansen 1981, Vesk and Borowitzka 1984, Wolkerling 1988, Wilson 1993, Agee 1995, Broadwater & Agee 1995, Kamas 1995 Lapointe 1995, Griffin & Broadwater 1996, Mays et al. 1996)

Table 4

General Sporangial Development Pattern For the Corallinaceae

	Stage 1	Stage 3		
		A	B	C
CLEAVAGE	Initiated	Arrested	Arrested	Arrested
CELL WALL	None	Spore Wall	Spore Wall	Spore Wall
CHLOROPLASTS	Mature	Immature	Immature	Mature
VACUOLES	Very	Diminished	Diminished	None
STARCH	None	Production	Parallel ER Stacks	Dispersal
VESICLE				
distribution	None	Production	Clumped	Dispersal
type	None	Osmiophillic	Osmiophillic	Osmiophillic

Table 5

Variable Sporangial Development Features in the Melobesioideae

	Stage 1	Stage 3		
		A	B	C
VACUOLES	**Many**	**Diminished**	**Diminished***	**None**
STARCH*	**None**	**Production**	**Parallel ER Stacks**	**Dispersed**
Lithothamnium		*S/V/ER*	*S/ER*	
Mesophyllum		*NA*	*S/ER*	
Clathroporphum		*NA*	*S/ER*	
Melobesia		*NA*	*S/ER*	
NUCLEUS				
shape	**Regular**	**Regular**	**Regular**	**Irregular**
association	**None**	**None**	**Present**	**Present/Absent**
Lithothamnium			*C/M*	*C/M*
Mesophyllum			*S*	*Absent*
Clathromorphum			*(ND)*	*(ND)*
Melobesia			*C/M*	*C/Mi*
VESICLE TYPES**	**None**	**Production**	**Variable**	**Variable/None**
Lithothamnium		*g*	*st*	
Mesophyllum		*c, g*		
Clathromorphum		*c, g*		
Melobesia		*c, g*	*st*	

C: chloroplast, c: cored vesicles ER: endoplasmic reticulum, g: granulose, M: mitochondria, NA: no association,
ND: not determined, S: starch, st: smooth textured vesicles
* timing differs in Lithothamnium (vacuoles persist to late Stage B)
**most variable aspect of Sporogenesis

Diagram 1

Formation of Secondary Pit-connections

(Johansen 1981)

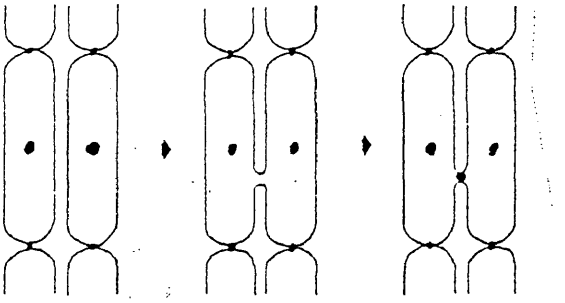


Diagram 2

Formation of Cell Fusions

(Johansen 1981)

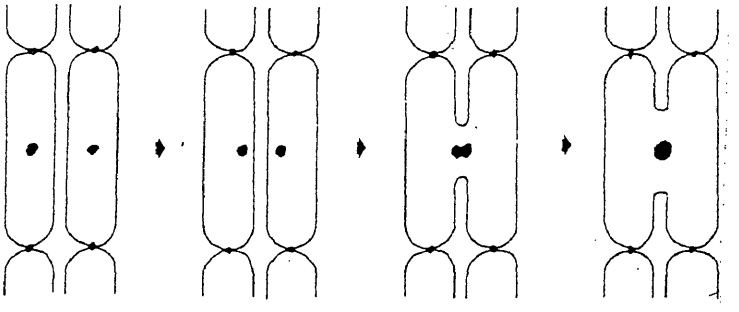


Diagram 3

Triphasic Life History
(Johansen 1981)

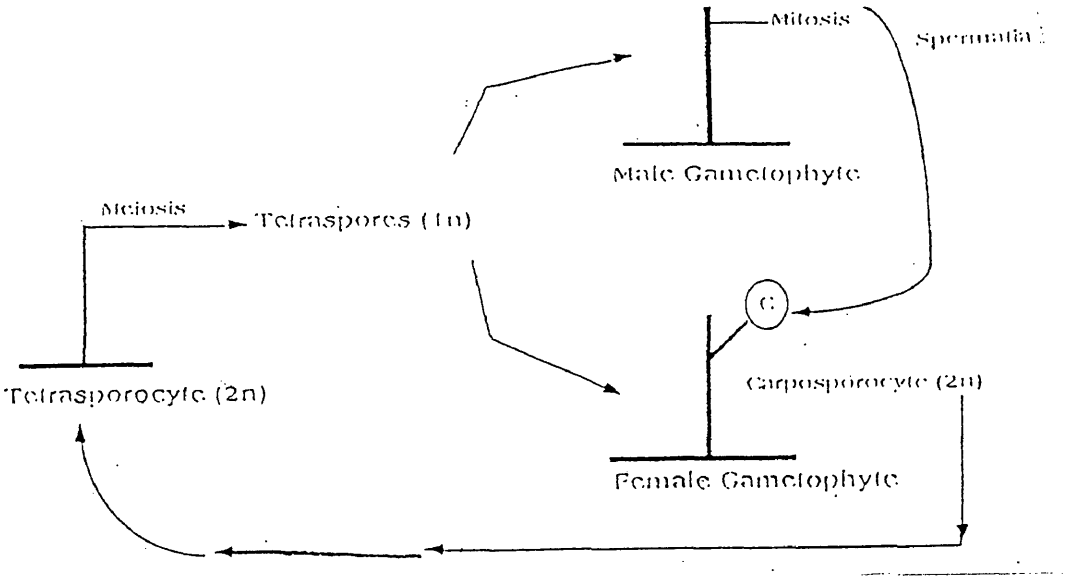


Diagram 4

Monomerous Thallus Constrcution
by Harvey, A.S. (adapted from Woelkerling 1988)
http:// www.botany.umc.ac.za/clines.htm

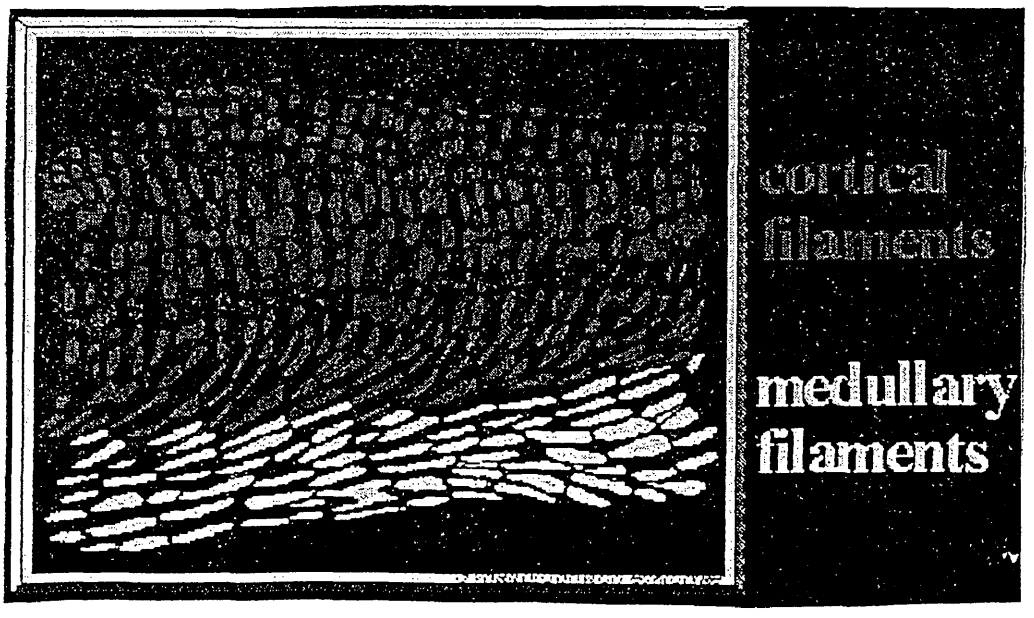
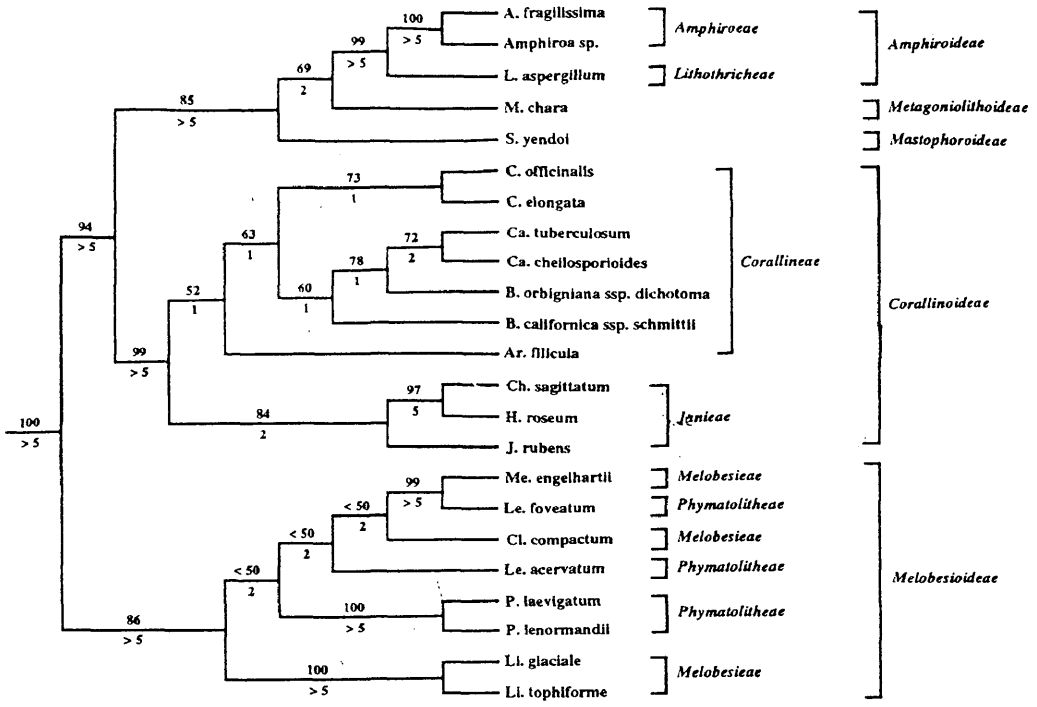


Diagram 5

18 s Ribosomal Molecular Data
(according to Baliey and Chapman 1996)



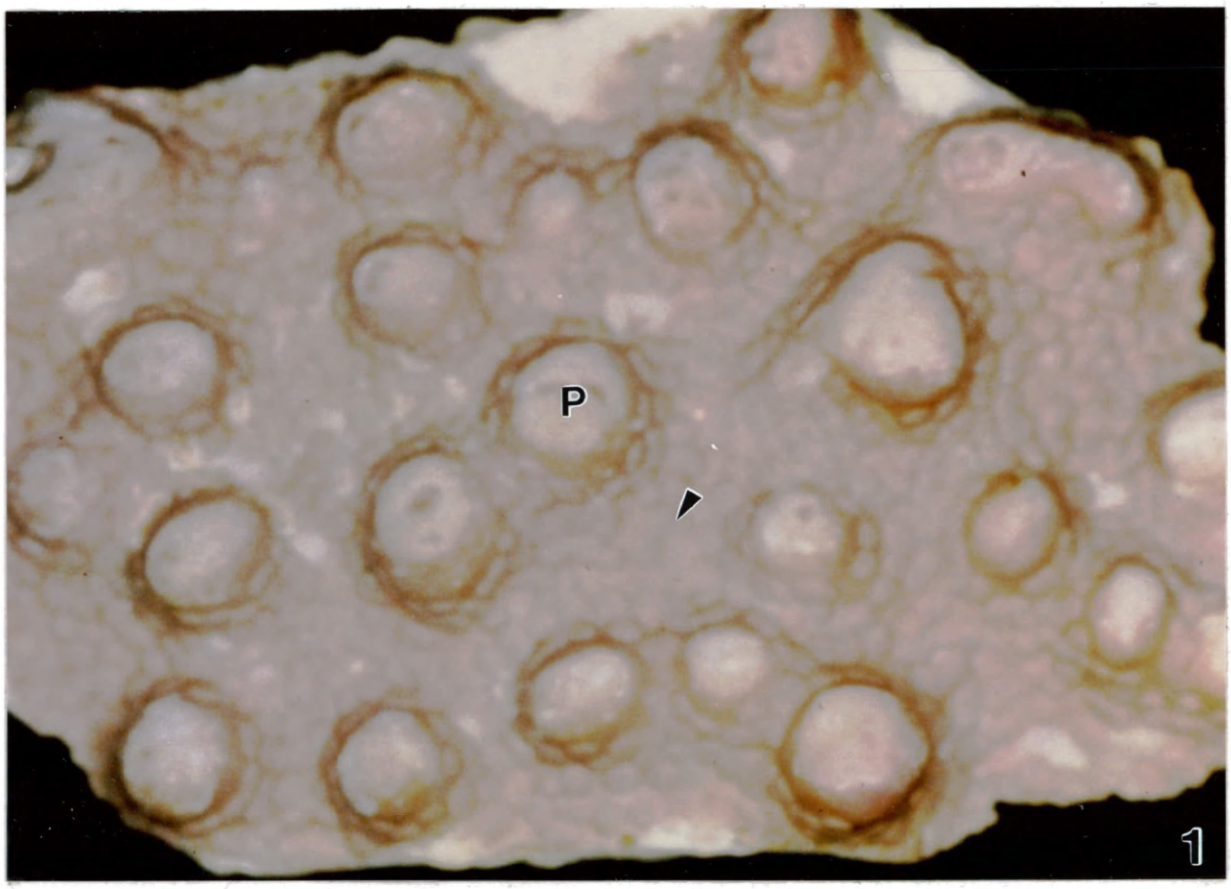
KEY TO ABBREVATIONS

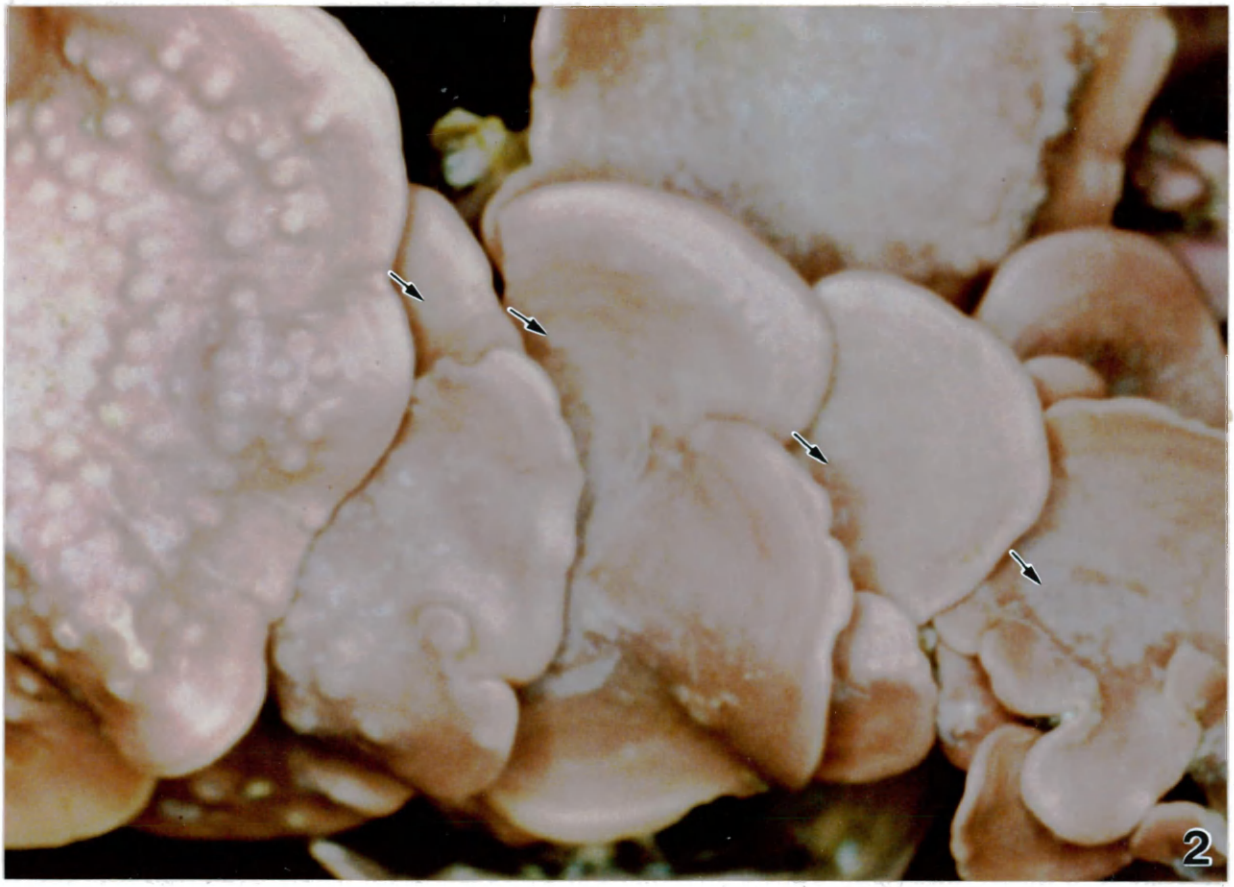
B, bottom
C, core
c, chloroplast
d, dark-cored vesicle
g, Golgi
i, intact cell
ig, irregular granular vesicle
L, lipid globule
l, large-cored vesicle
M, medulla
m, mitochondria
n, nucleus
no, nucleolus
o, osmiophillic vesicle
P, protuberance
rg, regular granular vesicle
S, stalk cell
s, starch
st, smooth-textured vesicle
T, top
v, vacuole
W, wall ring

Figures 1-2. Light Micrographs of General Thallus Structure

Figure 1. *Lithothamnium phymatoduem*'s pink continuous crust. Except on protuberances (P), conceptacles crowd the crust (arrowhead).

Figure 2. *Mesophyllum lamellatum* growing on an articulated coralline. Note pink crusts overlaping in a shingle like construction (arrows).


P
1


2

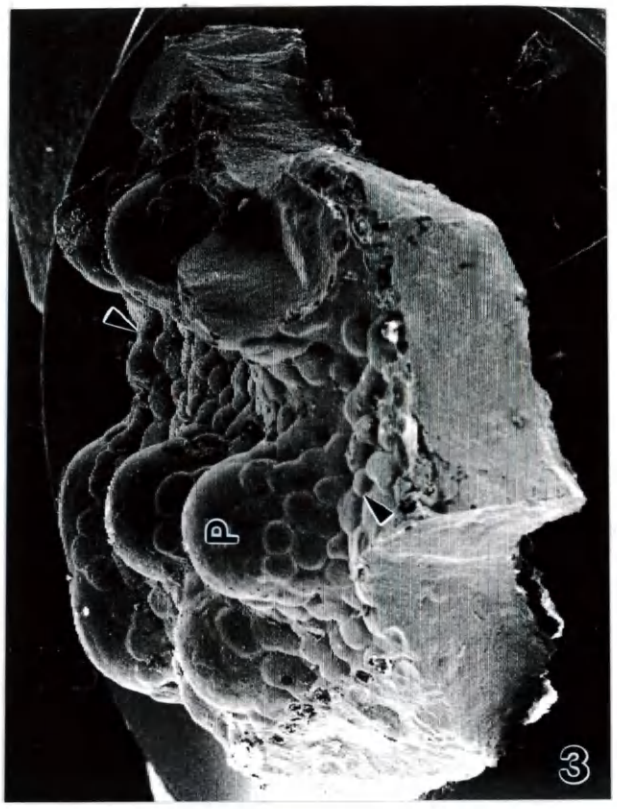
Figures 3-6. Scanning Electron Micrographs of General Thallus and Bisporangial Conceptacle Construction in *Lithothamnium phymatodeum*

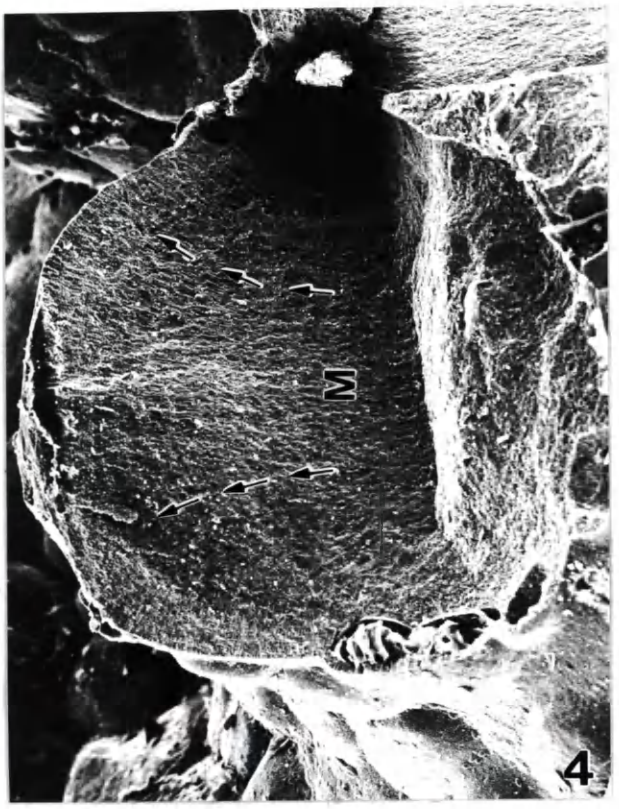
Figure 3. Monomerous thallus with patches of bisporangial conceptacles (arrowheads) present on crust, except at protuberances (P). x 9.

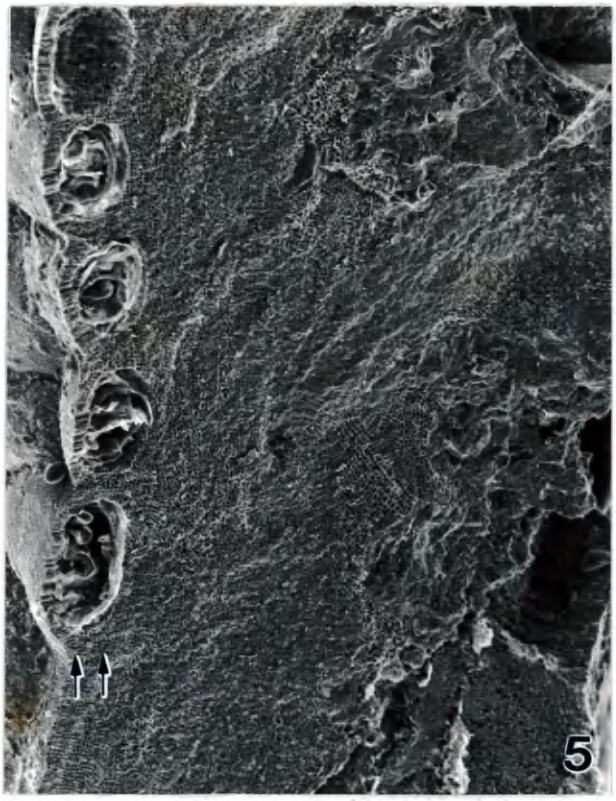
Figure 4. Longitudinal section through a protuberance. The medulla (M) consists of a core of coaxial filaments and the cortex (arrows) is composed of distal filaments radiating upwards towards the surface. x 274.

Figure 5. Row of longitudinally cut conceptacles (double arrows) at the surface. x 36.

Figure 6. Raised multiporate bisporangial conceptacle with pore plugs (arrows) denoting the presence of bisporangia. x 48.


P
3


M
4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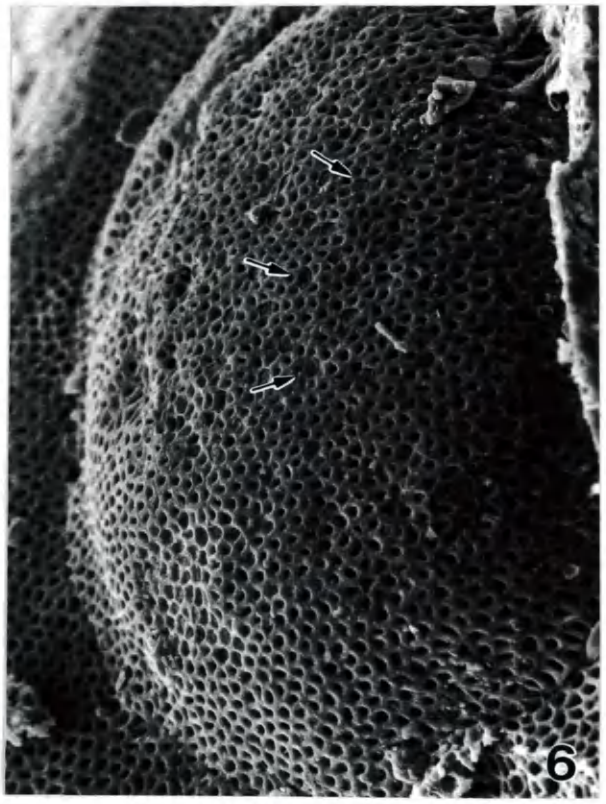
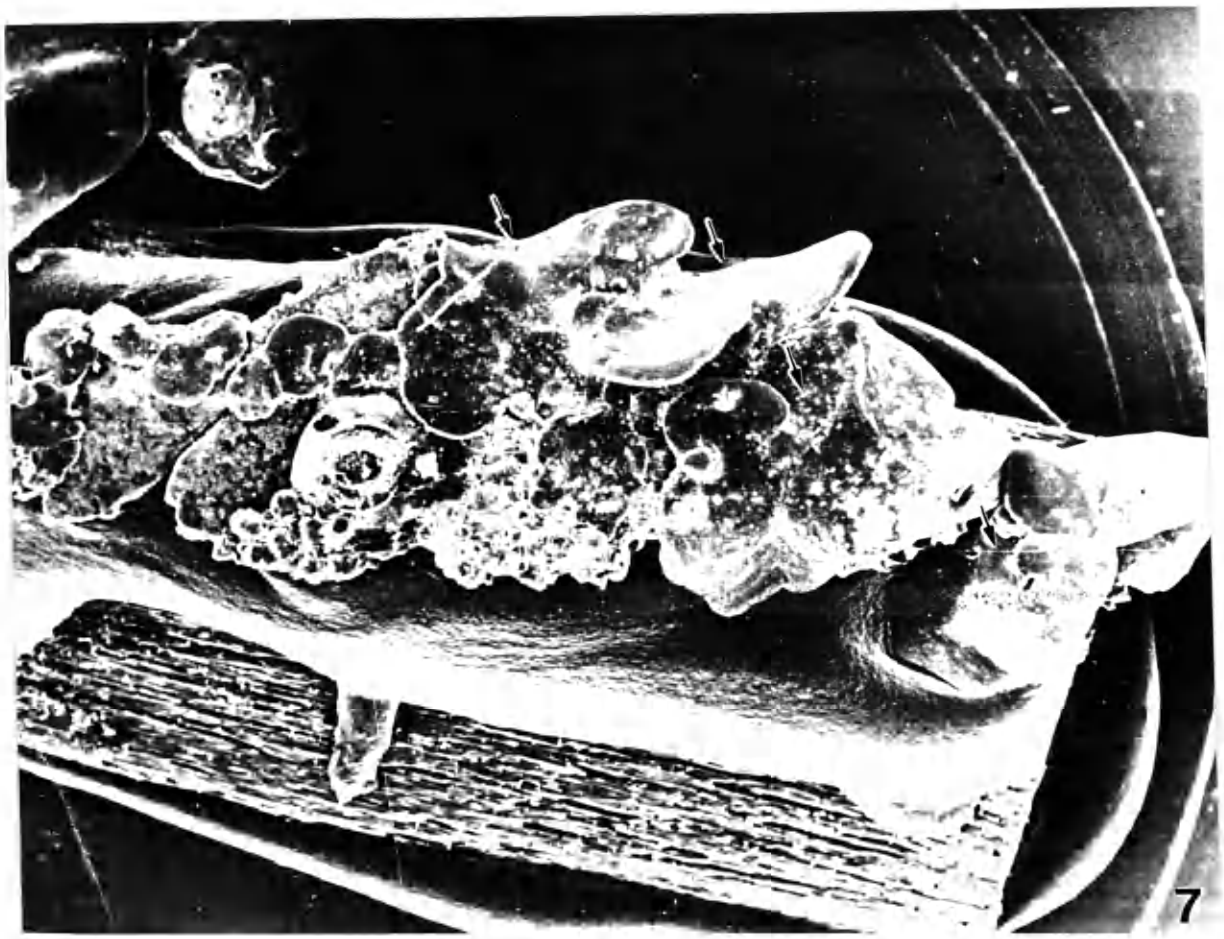

6

Figures 7-9. Scanning Electron Micrographs of General Thallus and Tetrasporangial Conceptacle Construction in *Mesophyllum lamellatum*

Figure 7. Thallus consisting of overlapping crusts with smooth-appearing surfaces (arrow). x 16.

Figure 8. Multiporate tetrasporangial conceptacles with pore plugs (arrows) are visible on the flattened conceptacle. x 17.

Figure 9. A longitudinal section through the monomerous crust of *Mesophyllum*. Note row of three conceptacles (arrowhead), one with tetraspores still inside (arrow). x 85.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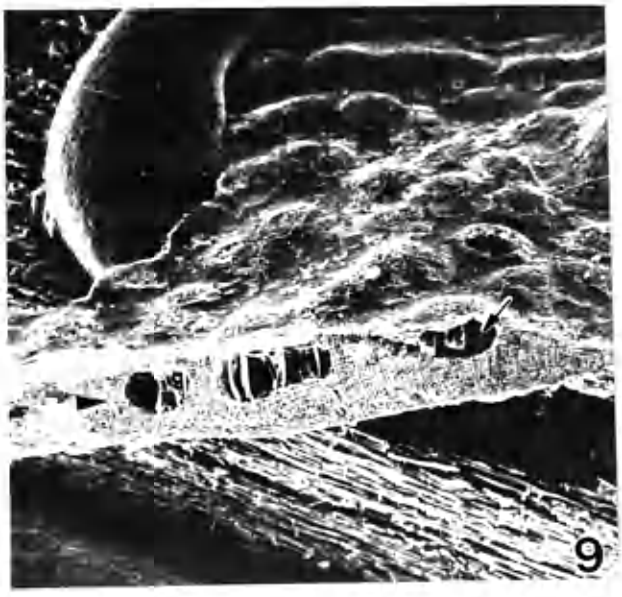
8


9

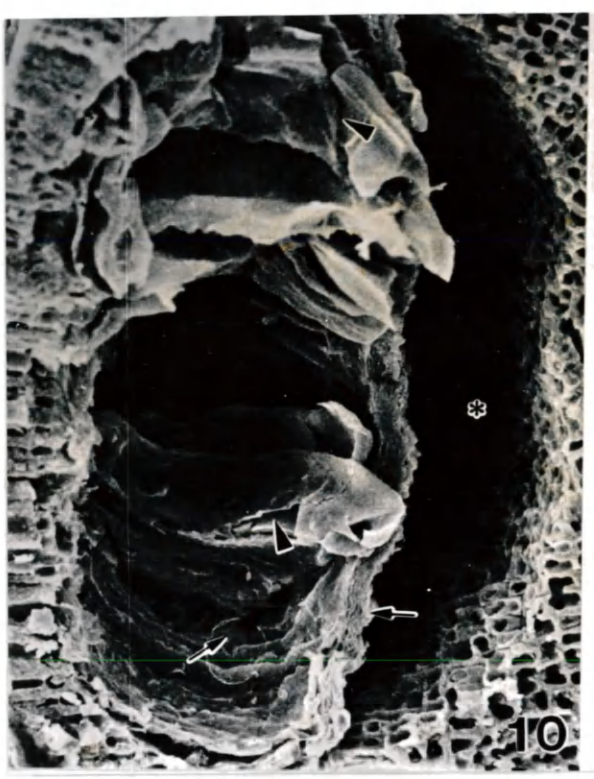
Figures 10-13. Scanning Electron Micrographs of Exterior Paraphyses Structure of *Lithothamnium phymatodeum.*

Figure 10. Longitudinal section of conceptacle showing paraphysis structure (arrows). Due to drying, the paraphysis has lifted off the conceptacle chamber floor (asterisk). Sheaths of paraphysis cells (arrowheads) also encase developing sporangia. x 300.

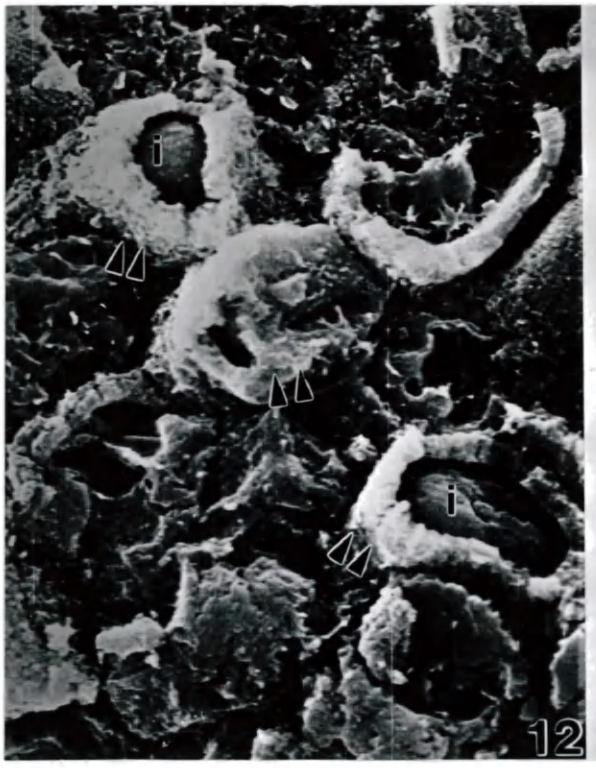
Figure 11. Exterior paraphysis structure. The paraphysis encapsulates conceptacle contents. x 224.

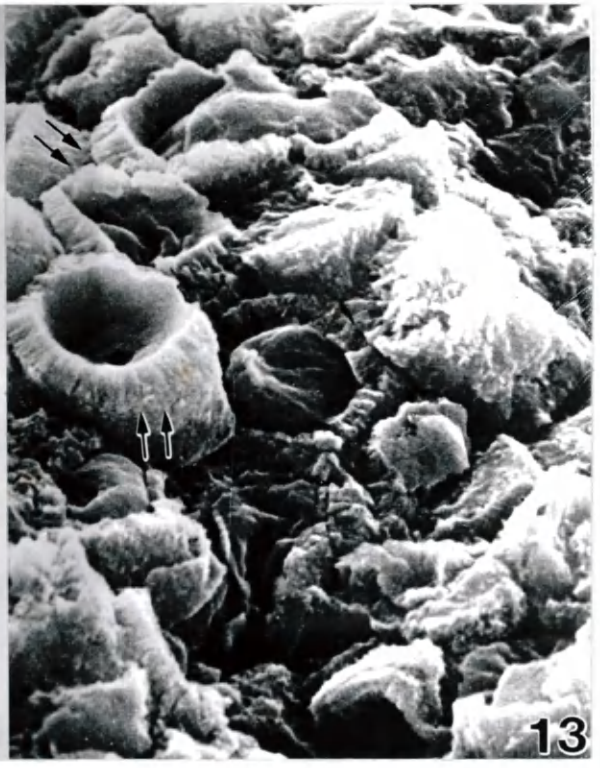
Figure 12. Higher magnification of exterior paraphysis structure. Calcium walls (double arrowheads) and intact cells (i) are either the outermost paraphysis cell layer or the innermost cells of the conceptacle which were pulled away from the conceptacle as the structure dried. x 1,441.

Figure 13. Exterior paraphysis cell layer showing striations (double arrows) indicating that the walls are embedded with calcium. x 2,729.


10


11


i
i
12


13

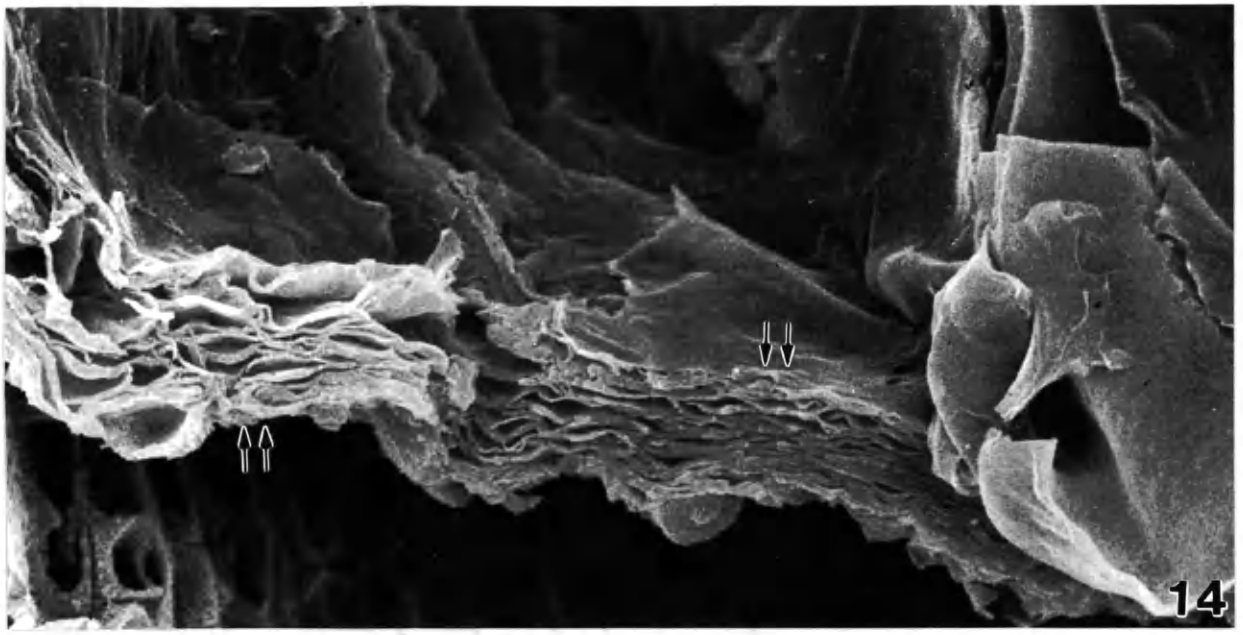
Figures 14-17. Scanning Electron Micrographs of Interior Paraphyses Structure in *Lithothamnium phymatodeum.*

Figure 14. Layers of paraphysis cells which normally line the bottom of the conceptacle (double arrows). Refer to Figure 10 for a view of the whole conceptacle. x 273.

Figure 15. Higher magnification of smooth inner layer of the paraphysis at bottom of the conceptacle of Figure 16. Pit connections are evident (arrowheads). x 1,310.

Figure 16. Longitudinal section through a conceptacle. Dried exterior paraphysis (arrow) lifted from the conceptacle. Inside conceptacle, cells of the paraphysis form a smooth inner coating (double arrowhead). x 202.

Figure 17. Greater magnification from Figure 16 of smooth inner paraphysis coating of walls of the conceptacle chamber (arrows). Individual cells of the paraphysis are not visible. Pit connections, (arrowheads). x 3,275.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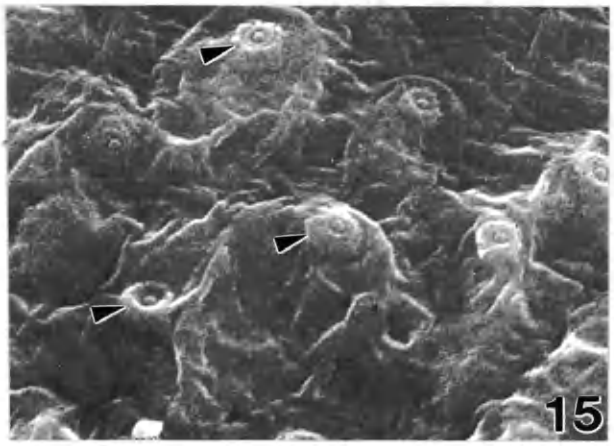
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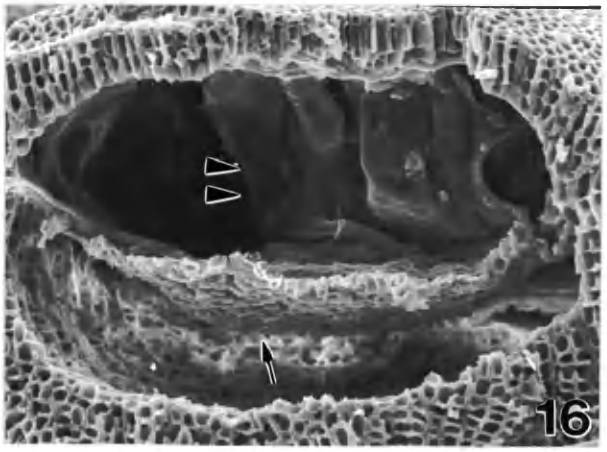

16

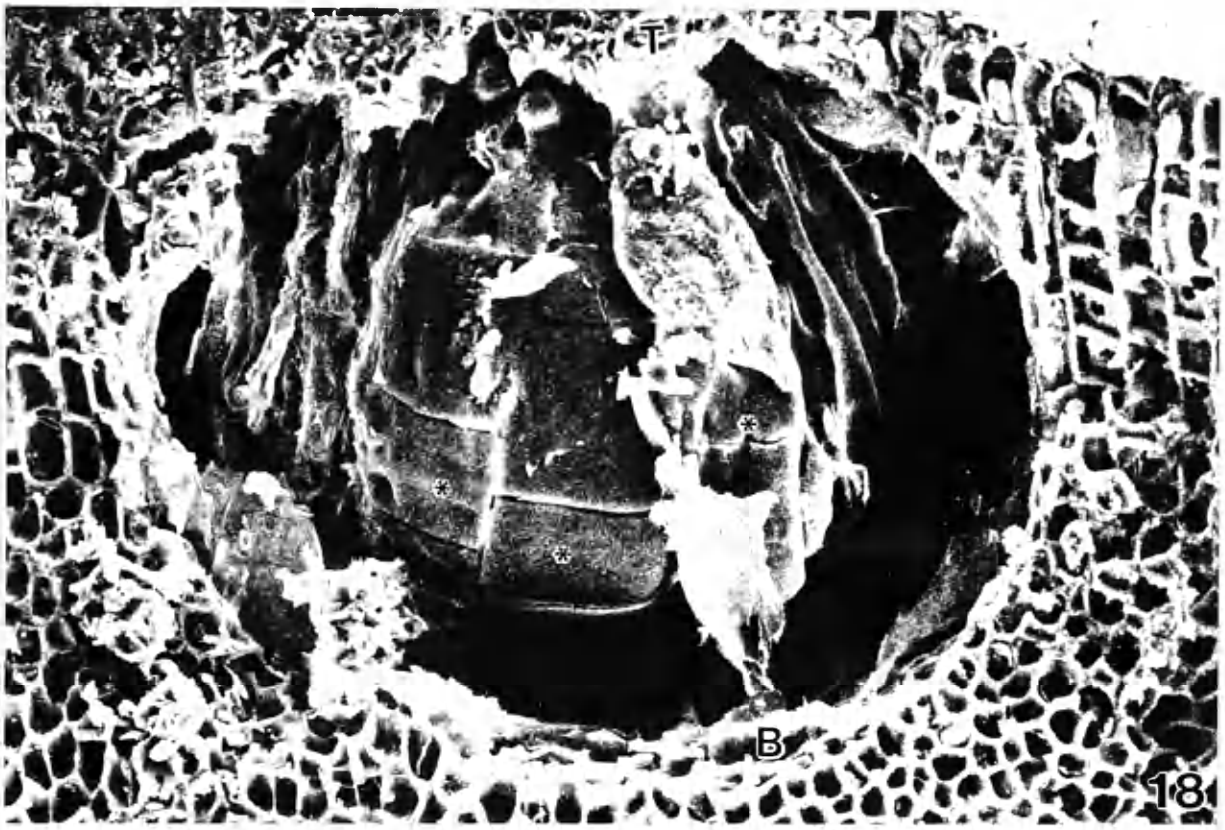

17

Figures 18-20. Scanning Electron Micrographs of Paraphysis Structure Surrounding Sporangia of *Mesophyllum lamellatum.*

Figure 18. Longitudinal section through a tetrasporangial conceptacle. Paraphysis cells encase sporangia and connect to both the inside top (T) and bottom (B) of conceptacle chamber. Also note developing tetrasporangia (asterisks). x 2,730.

Figure 19. Higher magnification of connection between paraphysis and conceptacle top (T). Many folds of paraphysis (arrows) material make it difficult to discern how the structure is connected to the conceptacle top (T). x 1,738.

Figure 20. Higher magnification of connection between paraphysis and conceptacle bottom (B). One paraphysis sheath (arrowhead) is shown connected to the bottom. x 873.


T
B
18


T
19


B
20

Figures 21-24. Transmission Electron Micrographs of Paraphysis cells of *Lithothamnium phymatodeum* (Figures 21-22) and *glaciale* (Figures 23-24).

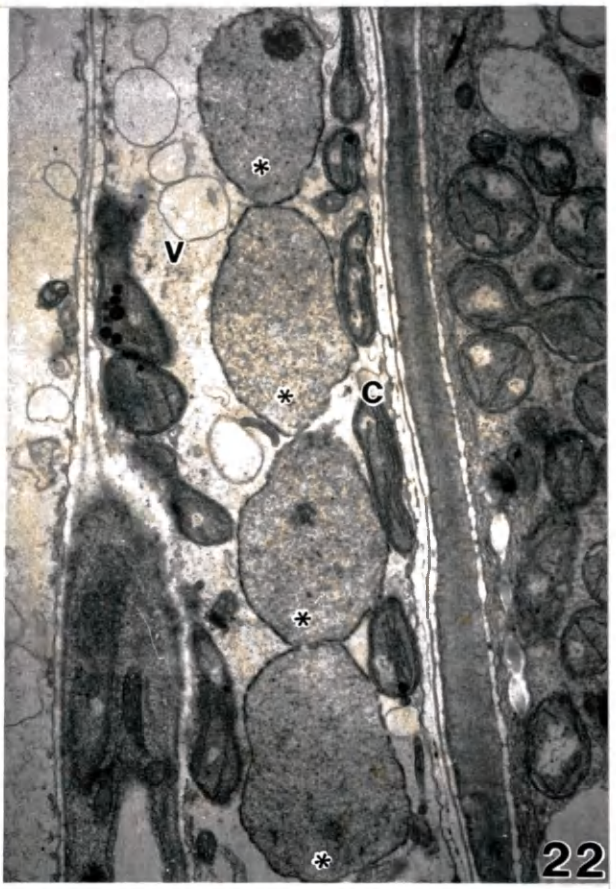
Figure 21. Longitudinal section through a conceptacle showing elongate paraphysis cells (arrow) located adjacent to developing bisporangium. Paraphysis' cells are very vacuolate (v) and have few cytoplasmic organelles but are frequently multinucleate (arrowhead). x 1,098.

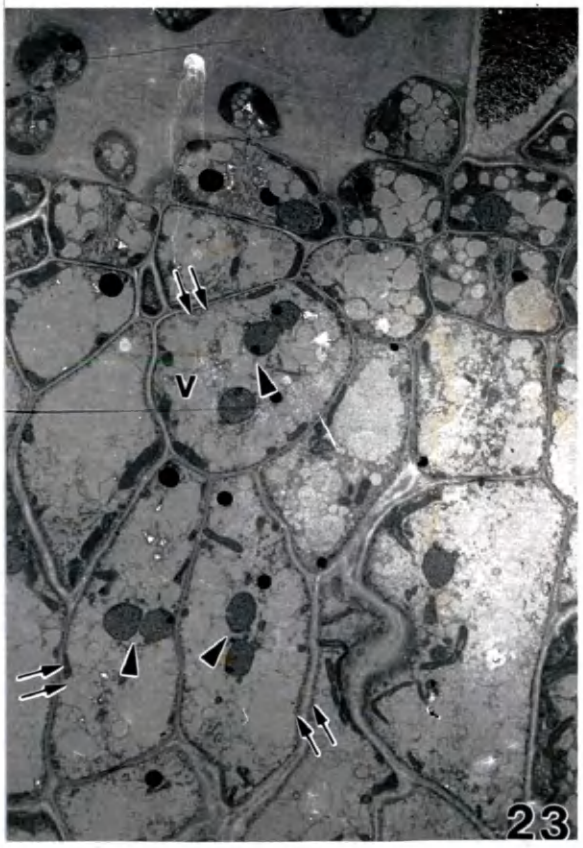
Figure 22. Higher magnification of the multinucleate cell from Figure 21. Four nuclei (asterisks) are present. Vacuoles (v); Chloroplasts with genophores (c). x 8,280.

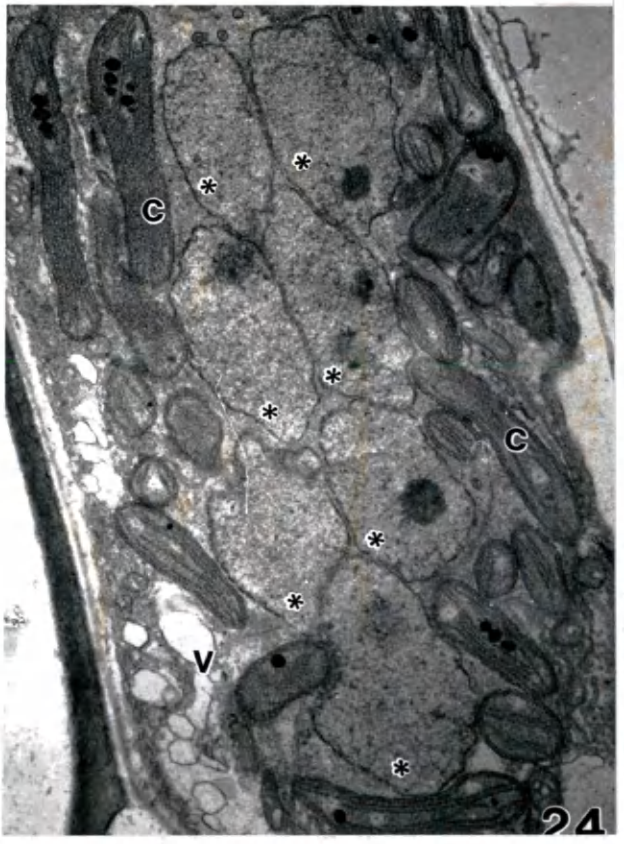
Figure 23. Truncate paraphysis cells (double arrows) located at top of conceptacle. Cells are vacuolate (v), have few organelles and are frequently multinucleate (arrowheads). x 1,098.

Figure 24. Paraphysis cell containing maximum observed nuclei within a sinlge section. Seven nuclei (asterisks) are centrally located in cell with more than average cellular contents. Chloroplasts (c); vacuoles (v). x 8,280.


V
21


V
C
22


V
23


C
C
V
24

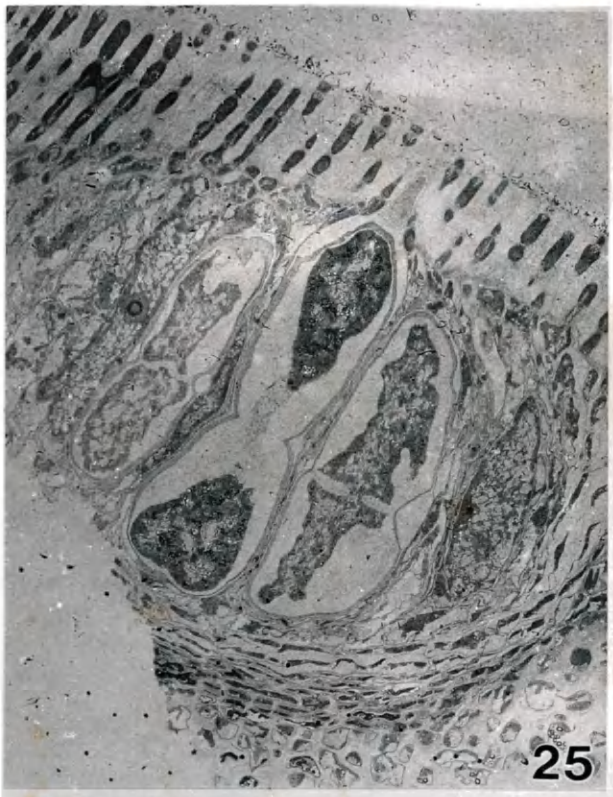
Figures 25-28. Transmission (Figures 25-26) and Scanning (Figures 27-28) Electron Micrographs of Sporangia

Figure 25. Longitudinal section through a *Lithothamnium phymatodeum* bisporangial conceptacle. Note asynchronous development of bisporangia. x 432.

Figure 26. Longitudinal section through a *Mesophyllum lamellatum* tetrasporangial conceptacle. Note asynchronous development and simultaneous zonate cleavage (arrows). x 360.

Figure 27. *Lithothamnium phymatodeum* bisporangium with stalk cell (asterisk). x 802.

Figure 28. *Mesophyllum lamellatum* tetraspore. x 772.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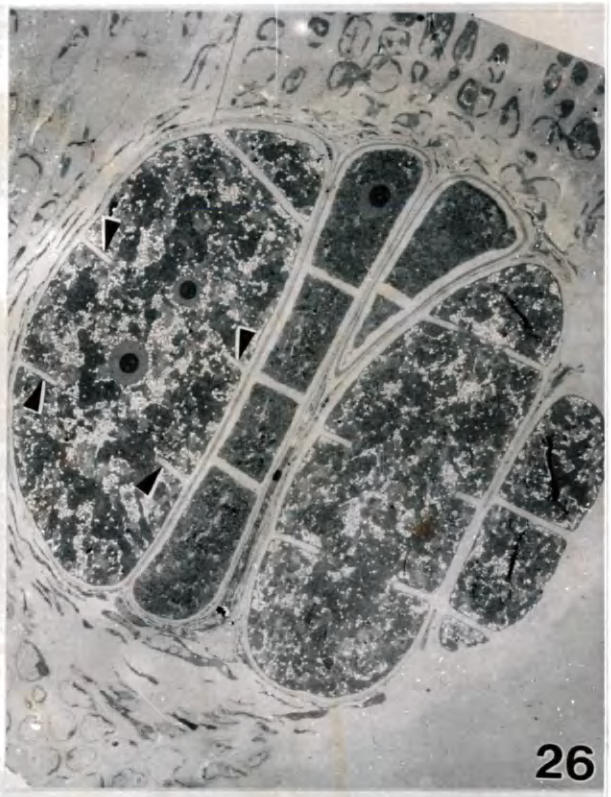
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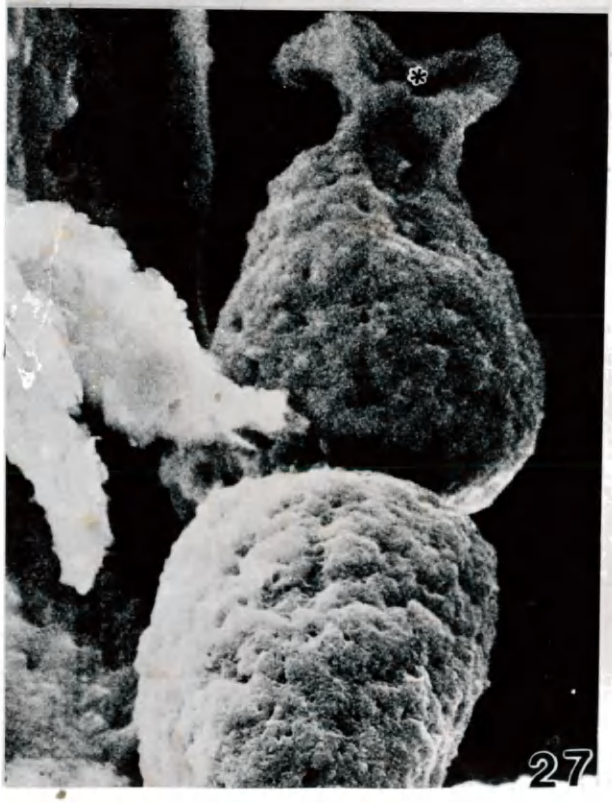
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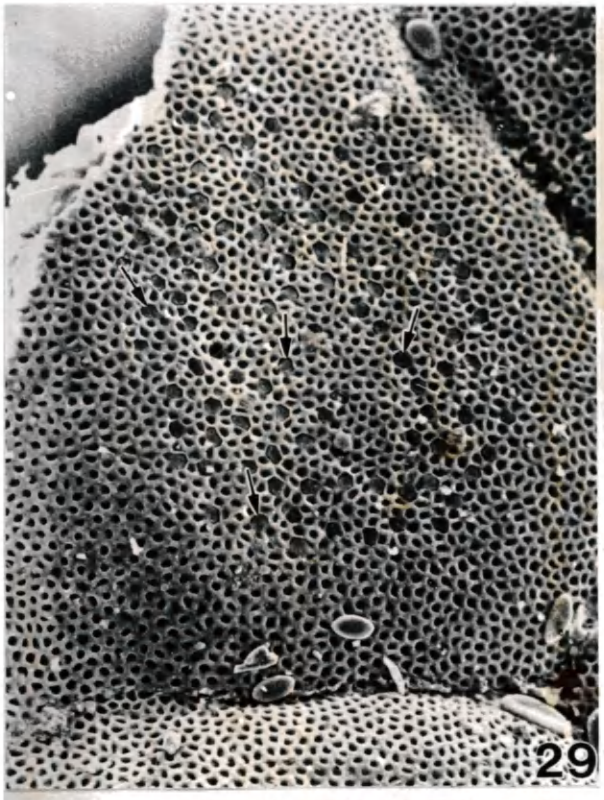
Figures 29-32. Scanning (Figures 29-30) and Transmission (Figures 31-32) Electron Micrographs of Pore Plugs and Related Features

Figure 29. Multiporate conceptacle of *Mesophyllum lamellatum*. Note presence of pore plugs (arrows). x 302.

Figure 30. *Mesophyllum lamellatum* tetrasporangium with pore plug (asterisk). x 592.

Figure 31. Longitudinal section through a conceptacle of *Lithothamnium glaciale* with two stage 1 bisporangia (b). One shows the presence of a pore plug (asterisk), while the other shows SER clumped (arrowhead) at the apical end of the bisporangium. x 1,515.

Figure 32. Higher magnification of clumped SER found in Figure 31. x 49,650.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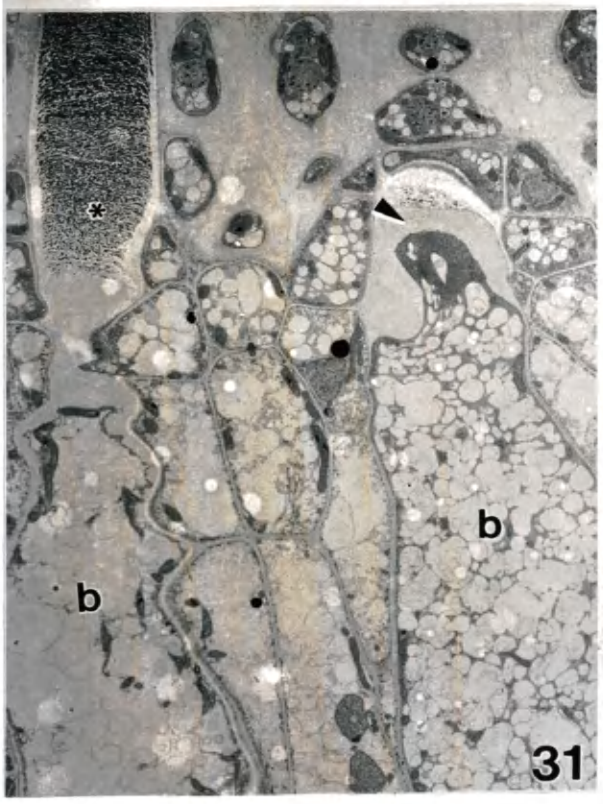

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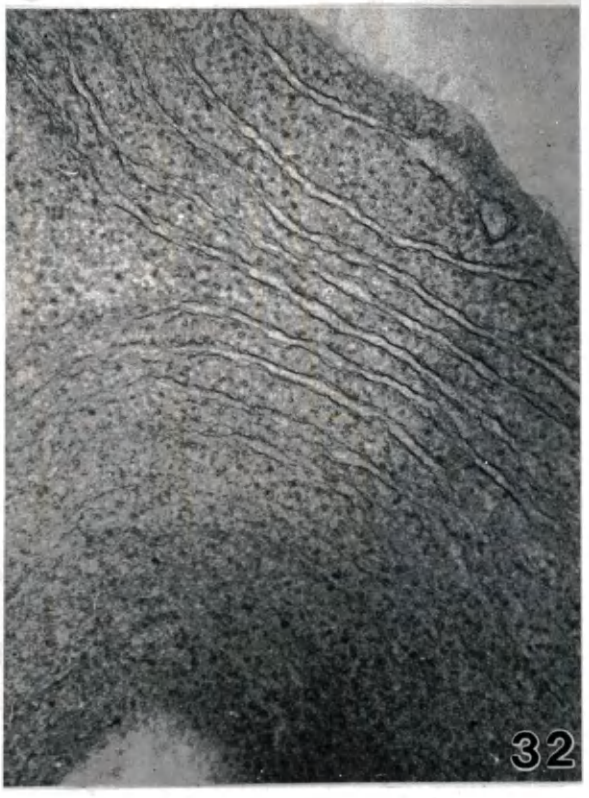
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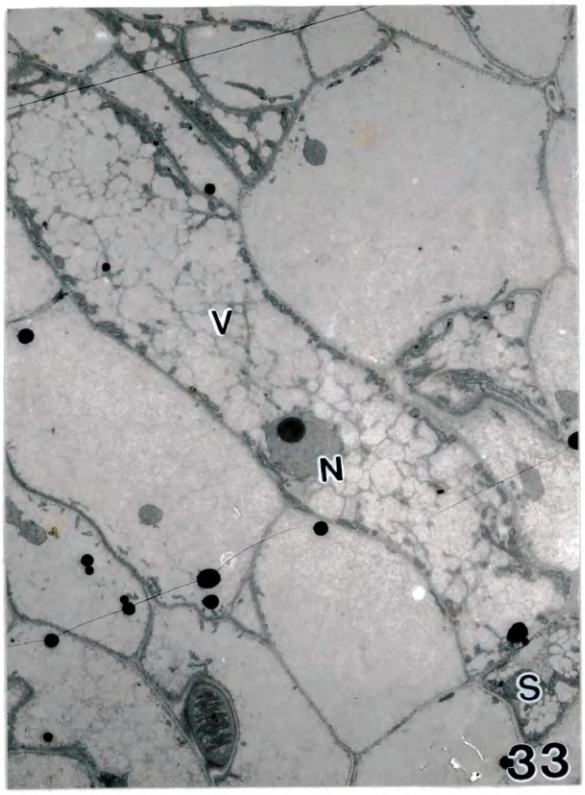
Figures 33-36. Transmission Electron Micrographs of Sporangia and Stalk Cells of *Lithothamnium phymatodeum.*

Figure 33. Stage 1 bisporangium with a stalk cell (S) attached. Nucleus (n); Vacuoles (v). x 915.

Figure 34. Higher magnification of stalk cell (S) present in Figure 33. Within stalk cell, a SER channel (asterisk) is evident. x 1,515.

Figure 35. Stage 3 bisporangium with increased cell wall deposition resulting in a wall ring (W) at basal end. Refer to Figure 52 for full bisporangium. x 1,515.

Figure 36. A different section of Figure 35 showing the pit connection (arrow) between stalk cell (S) and bisporangium. Wall ring (arrowheads). x 1,515.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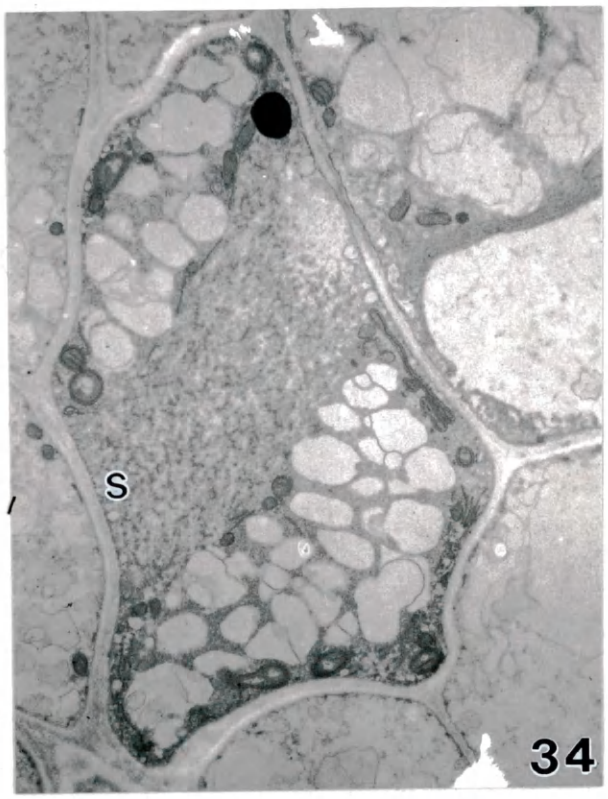
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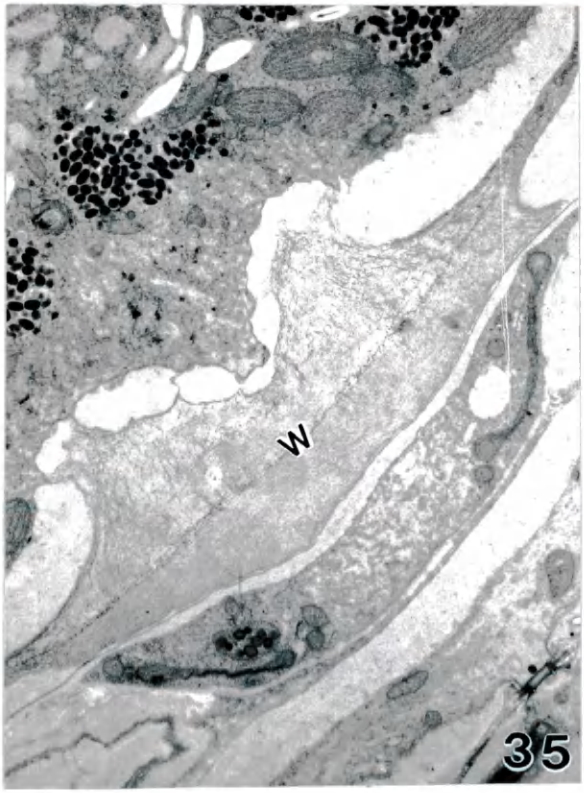
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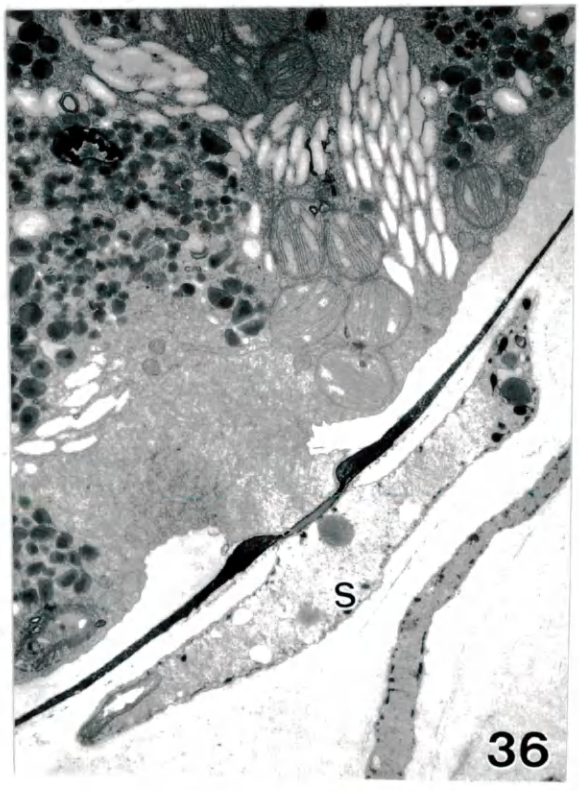
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Figures 37-41. Transmission Electron Micrographs of *Lithothamnium phymatodeum* (Figures 37-38,41) and *Mesophyllum lamellatum* (Figures 39-40).

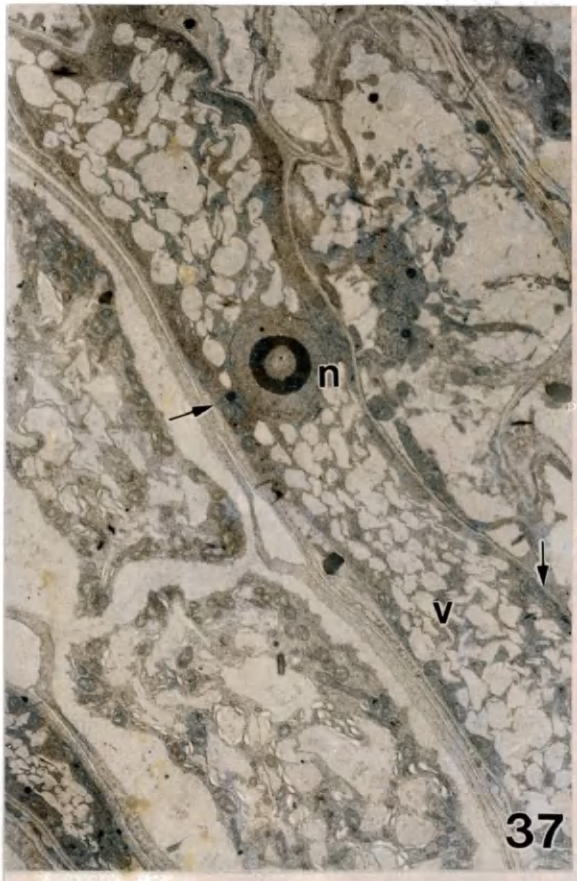
Figure 37. Stage 1 elongate bisporangium of *L. phymatodeum* having a centrally located nucleus (n), numerous vacuoles (v) and peripheral chloroplasts (arrows). x 1,515.

Figure 38. Higher magnification of nucleus (n) with ring shaped nucleolus (no) from Figure 37. Chloroplasts (c); vacuoles (v). x 1,515.

Figure 39. Stage 1 tetrasporangium of *M. lamellatum* with centrally located nucleus (n). The cell is very vacuolate (v) and has few organelles. Chloroplasts (arrows) are either located at the midregion or periphery of the tetrasporangium. x 6,900.

Figure 40. Stage 1 chloroplasts (c) of *M. lamellatum* with numerous genophores, a peripheral encircling thylakoid and 5-7 unstacked thylakoids. x 13,600.

Figure 41. Typical red algal association of Golgi (g), mitochondria (m), and ER (e). x 4,965.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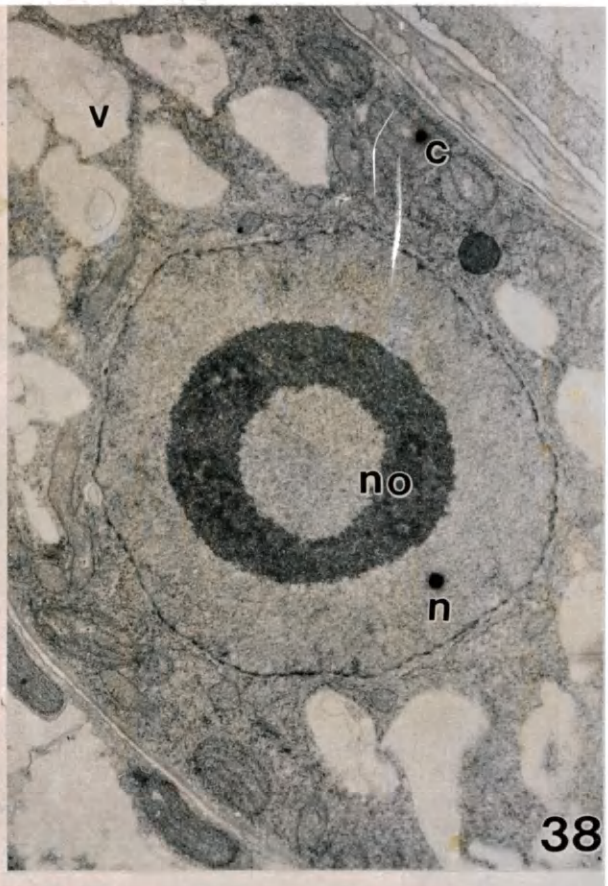
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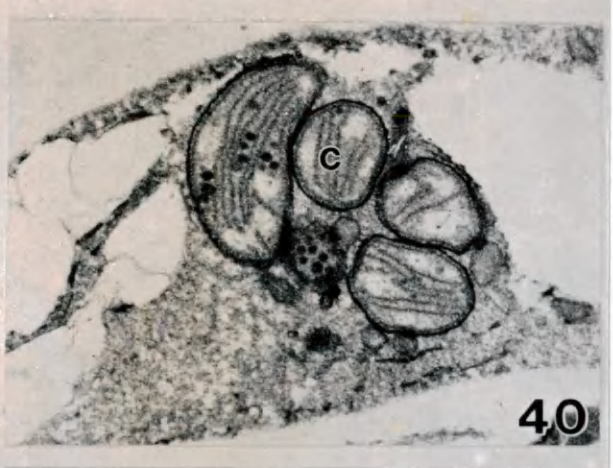
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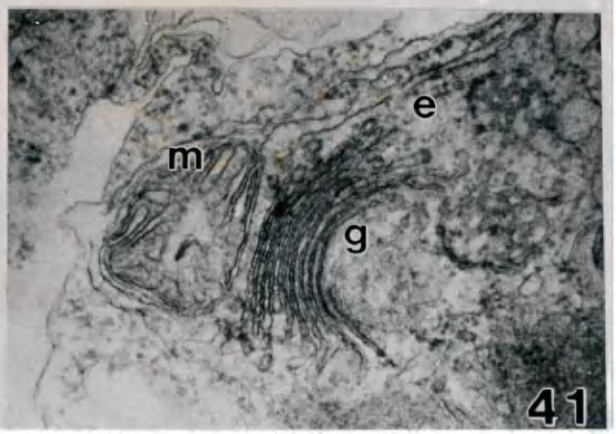
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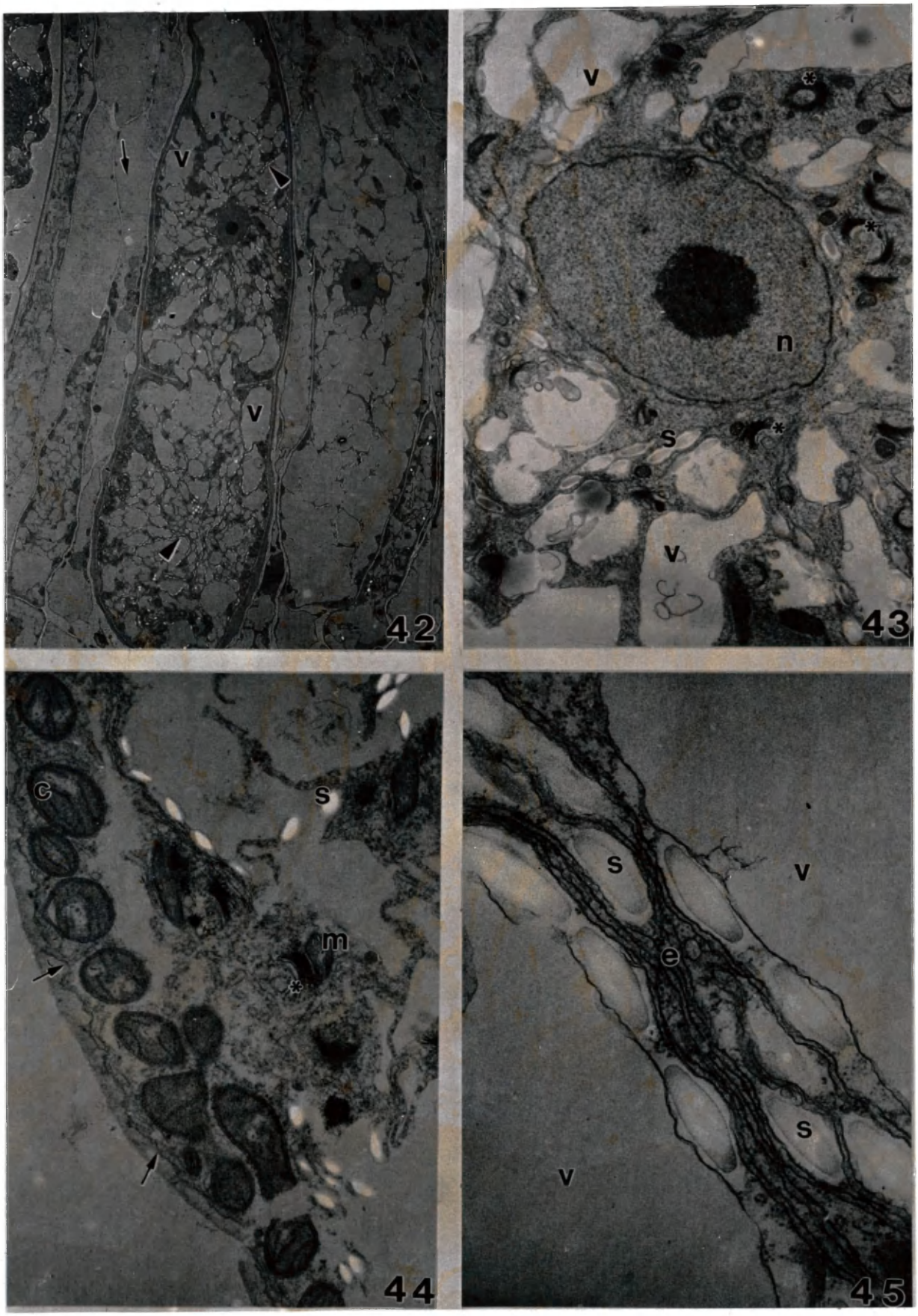
Figures 42-45. Transmission Electron Micrographs of Early Stage 3A Bisporangia of *Lithothamnium phymatodeum*.

Figure 42. Longitudinally cut bisporangium with starch (arrowheads) associated with ER or in between a vacuole (v) and an ER cisternum. Cell is still very vacuolate with most choloplasts at the mid-region or periphery of the bisporangium. Note elongate paraphysis cell (arrow). x 793.

Figure 43. Early stage 3A nucleus (n) with no organelle associations. Note increased numbers of Golgi (asterisks), vacuoles (v), and starch (s). x 5,980.

Figure 44. Chloroplasts (c) and ER (arrows) at periphery of an early stage 3A bisporangium. Chloroplast profiles have increased but have fewer thylakoids and genophores than Stage 1 bisporangia. Golgi (asterisk); mitochondira (m); starch (s). x 9,360.

Figure 45. Starch (s) sandwiched between a vacuole (v) and an ER cisternum (e) or surrounded on both sides by ER. x 29,120.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Figures 46-51. Transmission Electron Micrographs of Early Stage 3A Tetrasporangia of *Mesophyllum lamellatum.*

Figure 46. Longitudinal section of tetrasporangium with decreased numbers of vacuoles (v) commitant with production of dark-core vesicles (arrowheads) and osmiophillic vesicles (arrows). x 915.

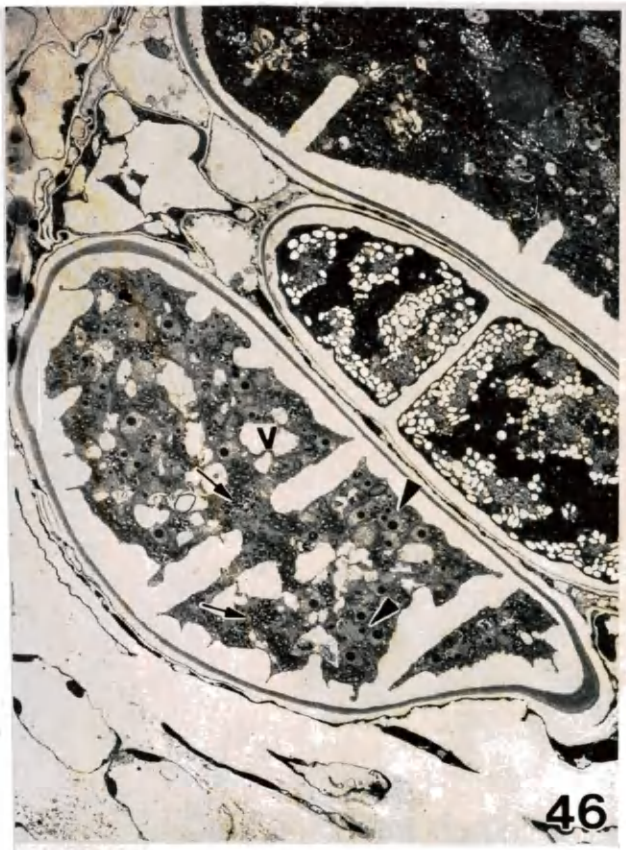
Figure 47. Tetrasporangial nucleus surrounded by large-cored vesicles (l), immature chloroplasts (c), and vacuoles (v). x 2,525.

Figure 48. Higher magnification of large-cored vesicles (l) in which the center core (C) occupies almost the entire volume of the vesicle (double arrow). x 16,120.

Figure 49. Cytoplasm of a early stage 3A tetrasporangium with immature chloroplasts (c) clumps of osmiophillic vesicles (o), and donut-like cored vesicles (arrowheads). x 5,980.

Figure 50. Higher magnification of dark-cored vesicles (d). Note concentric membranous material (asterisk) and osmiophillic vesicles (o). x 9,360.

Figure 51. Cytoplasmic contents of an early stage 3A tetrasporangium with vacuoles (v), immature chloroplasts (c), osmiophillic vesicles (o), and concentric membranous material (asterisks). x 4,420.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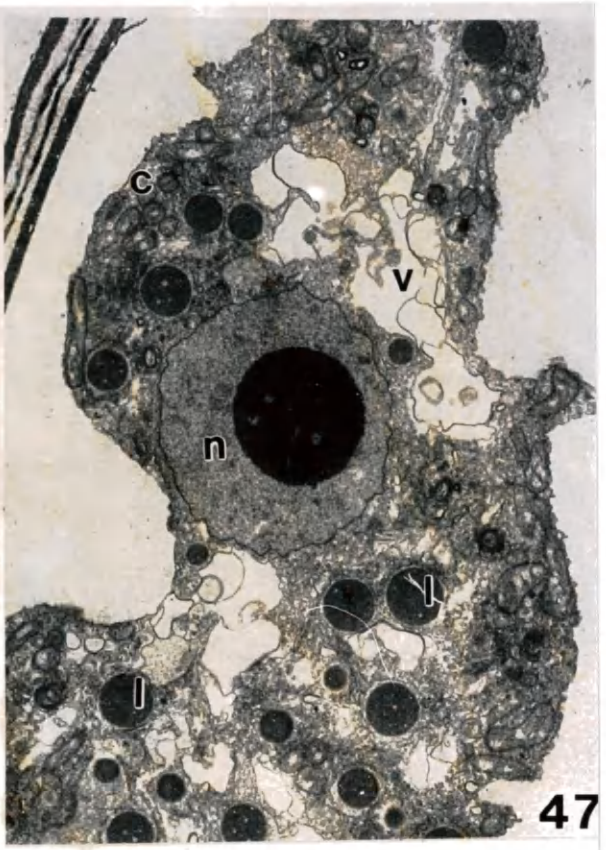
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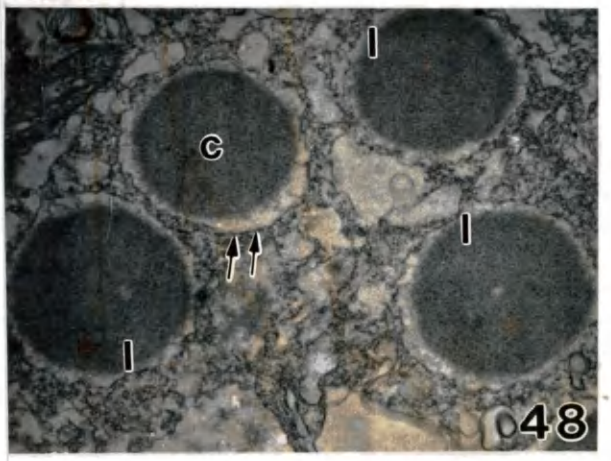
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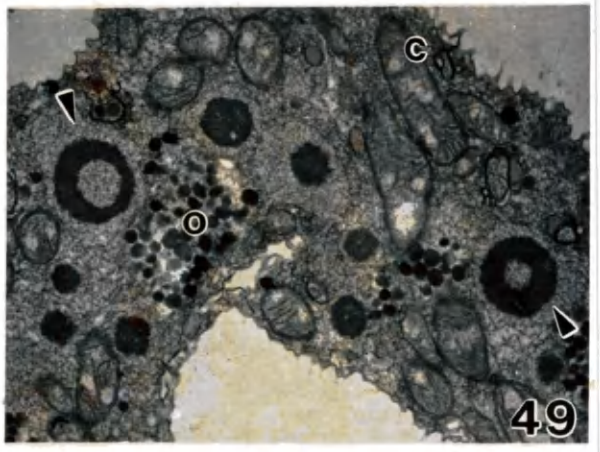
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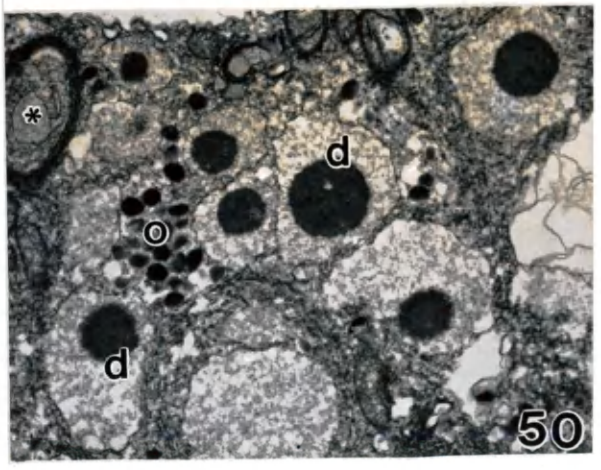
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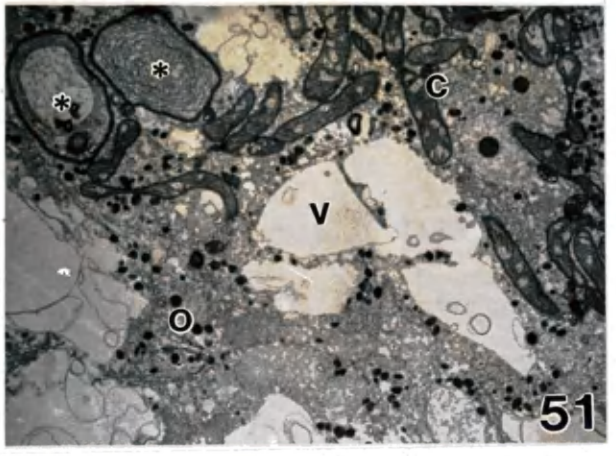
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Figures 52-56. Transmission Electron Micrographs of Late Stage 3A Bisporangia of *Lithothamnium phymatodeum.*

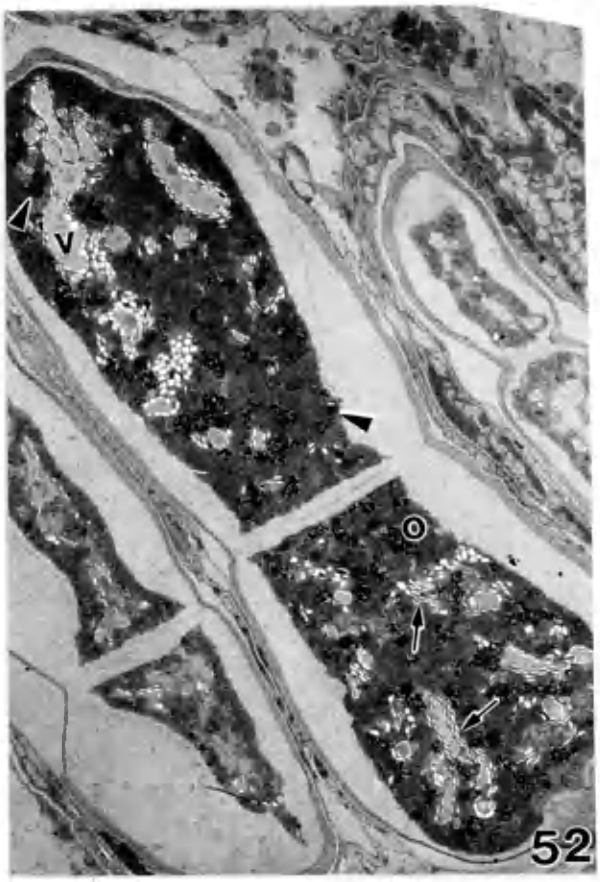
Figure 52. Bisporangium with very reduced numbers of vacuoles (v) and increased deposition of starch in parallel stacks (arrows) between ER cisternae. Osmiophillic vesicles (o) are clumped and many chloroplasts are present. Lipid globules (arrowheads) are evident. x 915.

Figure 53. Nucleus (n) with associated chloroplasts (c) and mitochondria (arrowheads). x 5,100.

Figure 54. Higher magnification of starch (s) organized as parallel stacks in association with diminished vacuoles (v). x 18,600.

Figure 55. Golgi body (g) production of smooth textured vesicles (st). Note the typical red agal mitochondria/ER/golgi association. x 49,650.

Figure 56. High magnification of irregular granular vesicles (ig). Starch (s); osmophillic vesicles (o). x33,600.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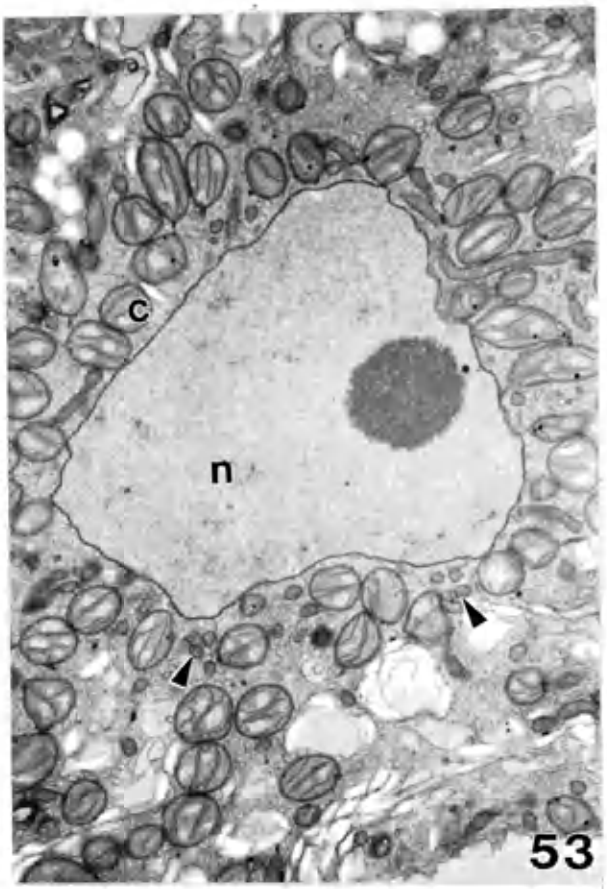
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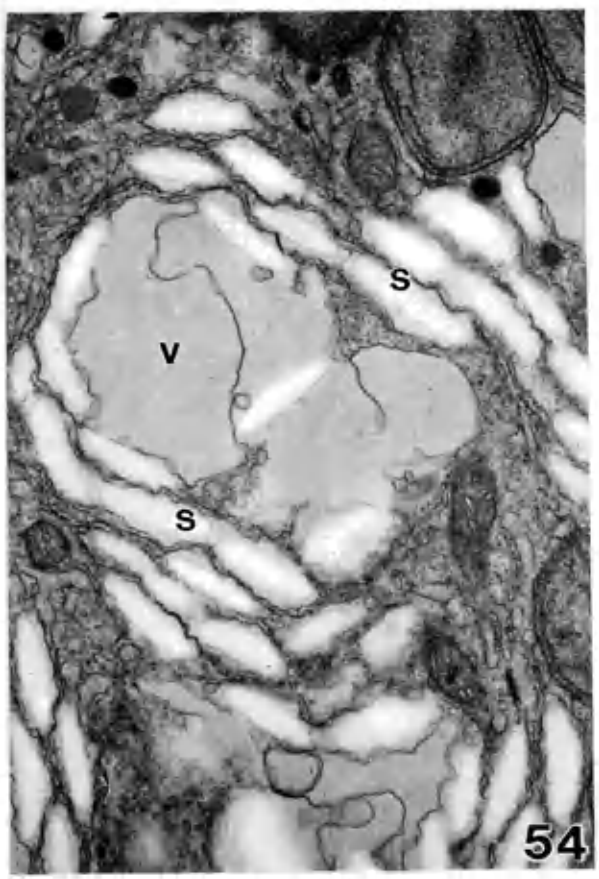
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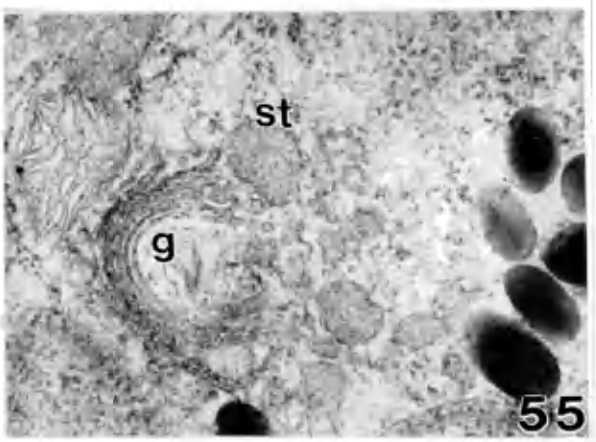
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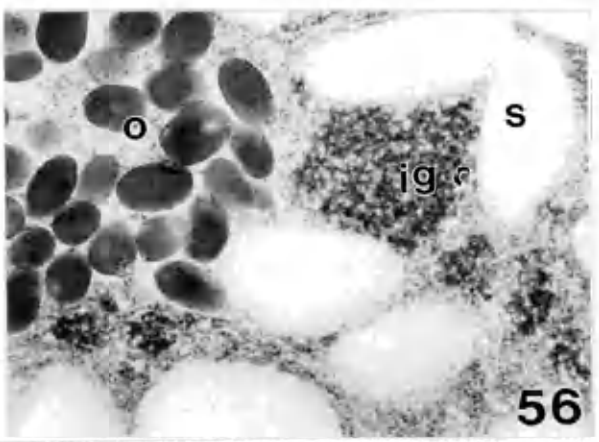
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Figures 57-61. Transmission electron micrographs of Late Stage 3A Tetrasporangia of *Mesophyllum lamellatum.*

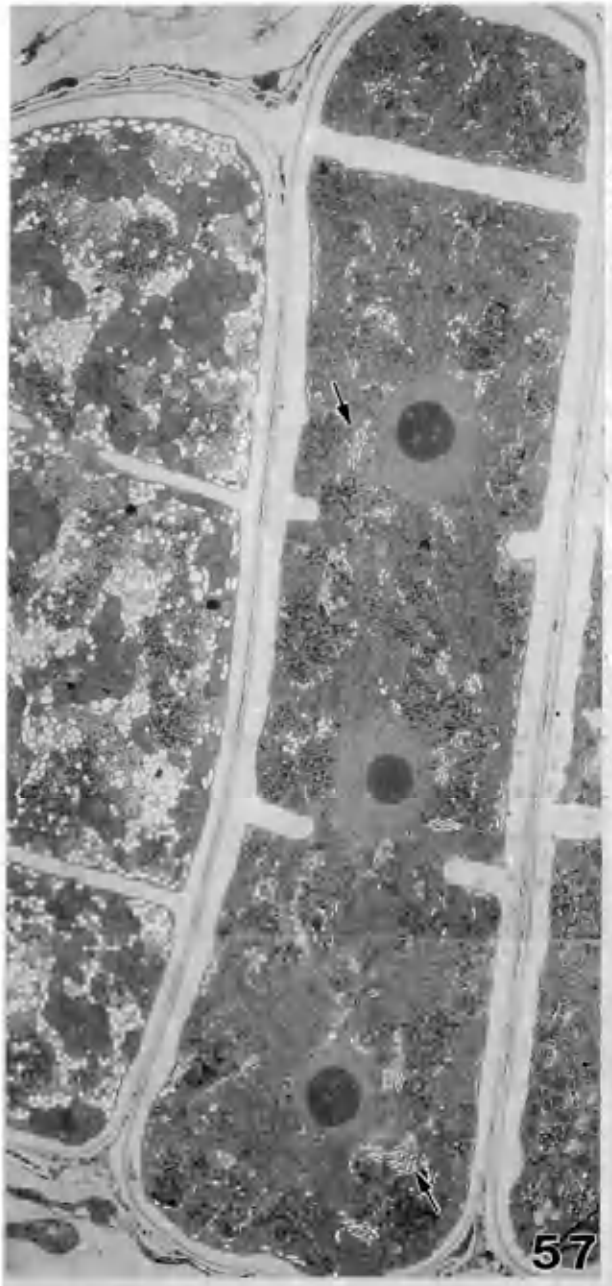
Figure 57. Longitudinal section of tetrasporangium with nuclei in nascent spores. Note parallel stacks of starch (arrows) and loss of vacuoles. x 976.

Figure 58. Higher magification of tetrasporangial nucleus (n) showing no organelle association. Chloroplasts (c); starch (s); mitochondria (m); osmiophillic vesicles (o). x 5,440.

Figure 59. Higher magnifaction of irregular granular vesicles (ig). x 33,600.

Figure 60. Higher magnificaton showing increased numbers of thylakoids in chloroplast (c). Note Golgi production of dark-cored vesicles (double arrows). Irregular granular vesicles (ig); osmiophillic vesicles (o). x 11,520.

Figure 61. Higher magnification of regular granular vesicles (rg). x 18,600.



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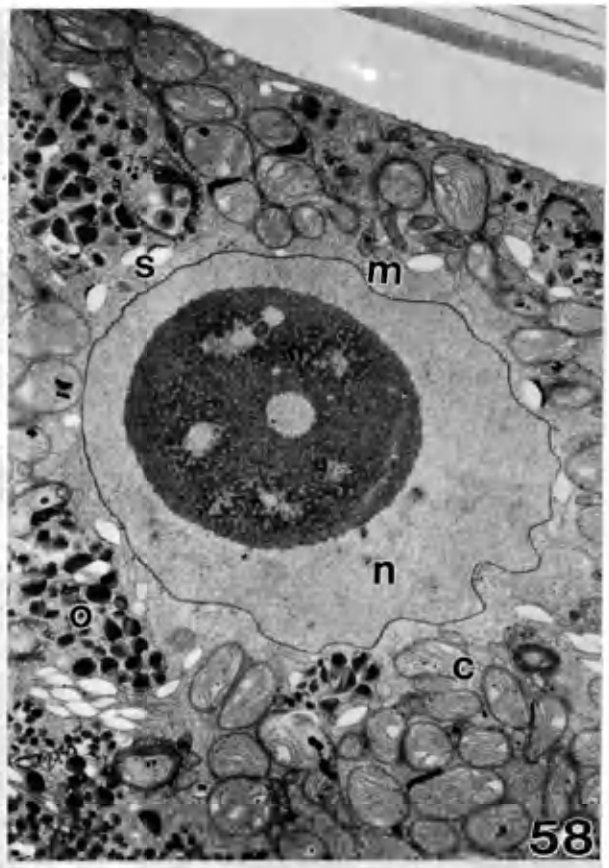

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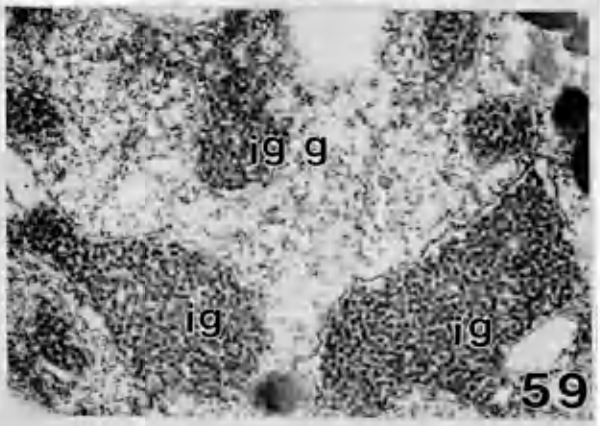

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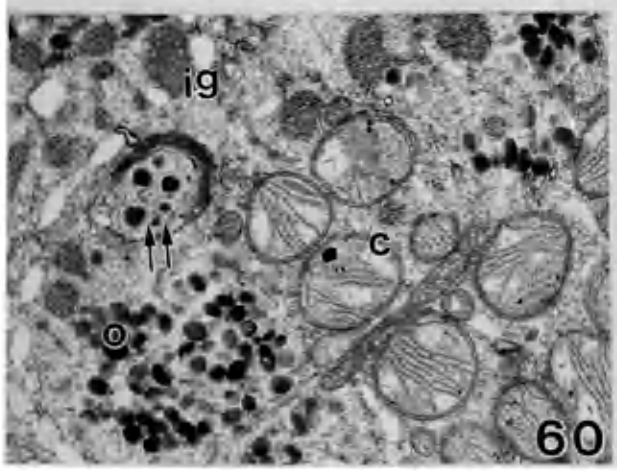

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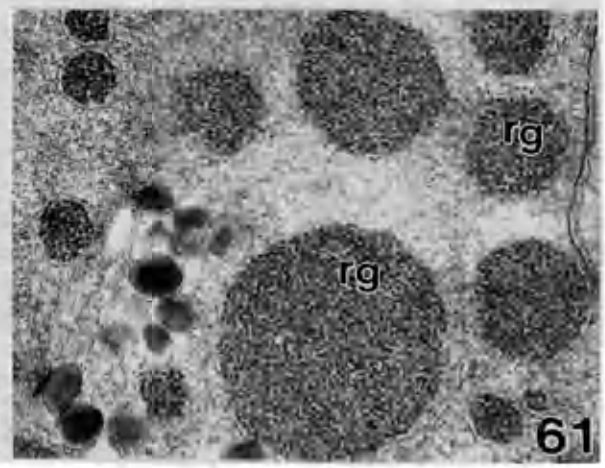

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Figures 62-66. Transmission Electron Micrographs of Stage 3B Bisporangia of *Lithothamnium phymatodeum.*

Figure 62. Bisporangium showing loss of vacuoles. Osmophillic vesicles (o) are clumped and parallel stacks of starch (s) are present. Lipid globules (asterisk). x 1,414.

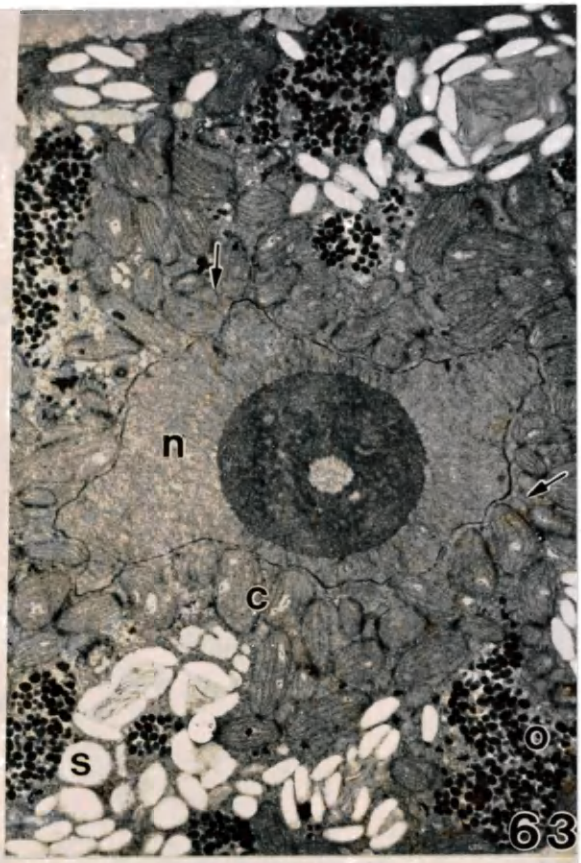
Figure 63. Nucleus (n) with numerous associated chloroplasts (c) and some associated mitochondria (arrow). Starch (s); osmophillic vesicles (o). x 5,100.

Figure 64. Higher magnification of mature chloroplasts (c) with electron dense structures (double arrows) between dividing lobes. x 18,600.

Figure 65. High magnification of lipid globules (L). x 26,800.

Figure 66. High magnification of parallel stacked starch (s) surrounded on both sides by ER cisternae (arrows). x 14,880.

62



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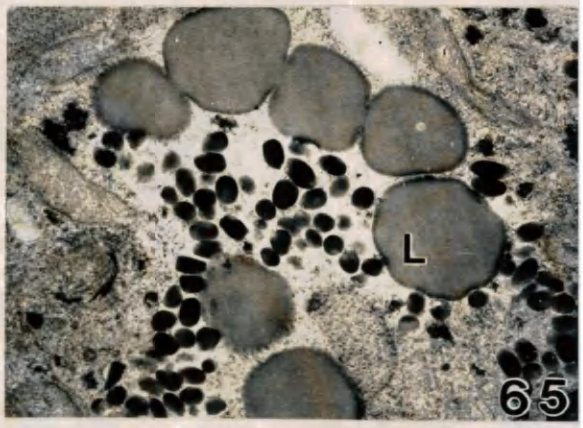

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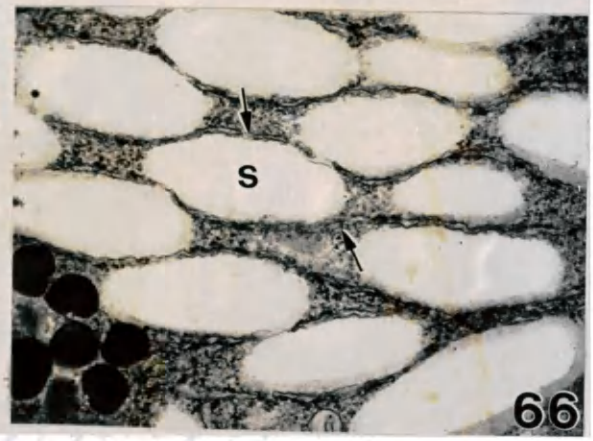

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Figures 67-71. Transmission Electron Micrographs of Stage 3B Tetrasporangia of *Mesophyllum lamellatum.*

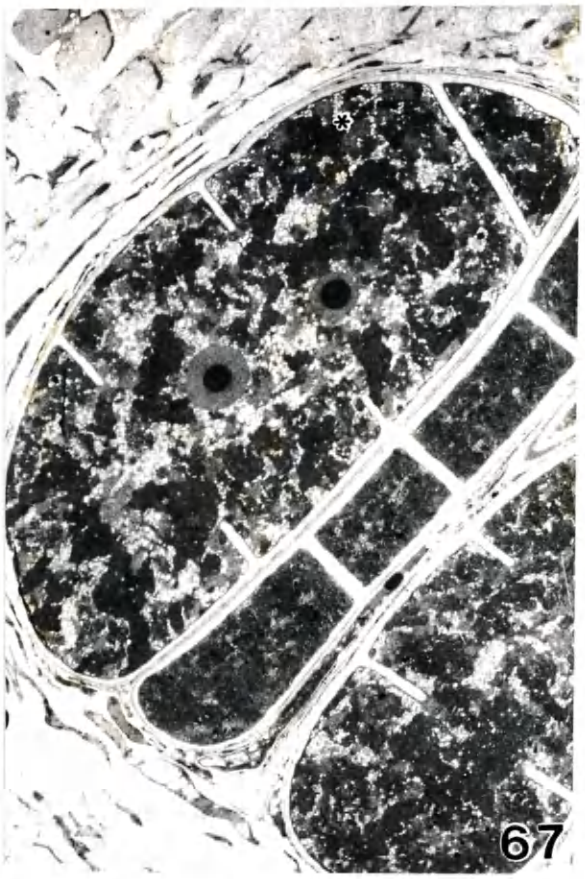
Figure 67. Conceptacle with stage 3B tetrasporangium (asterisk) showing simultaneous zonate cleavage. x 480.

Figure 68. Nucleus (n) with starch (s) association. Note Golgi (arrow) production of osmiophillic vesicles (o). Mature chloroplasts (c); mitochondria (arrowheads). x 4,080.

Figure 69. Tetrasporangium showing subdivision of cytoplasm into disparate regions for accumulation of starch (s), osmiophillic vesicles (o), and chloroplasts (c). x 732.

Figure 70. High magnification of cytoplasmic contents with starch (s), mature chloroplasts (c), and Golgi (g) production of osmiophillic vesicles (o). x 3,640.

Figure 71. High magnification of osmophillic vesicles to show difference in electron opacity. x 14,880.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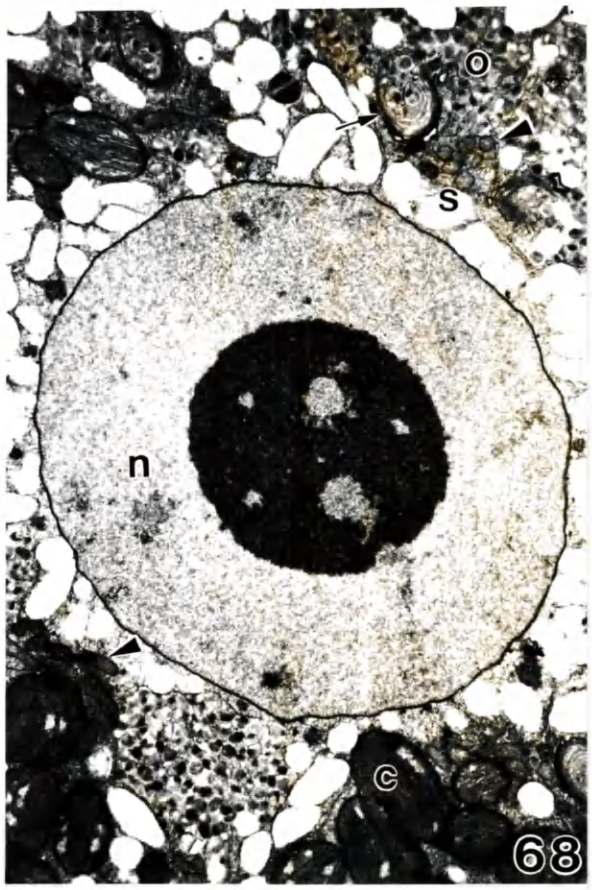
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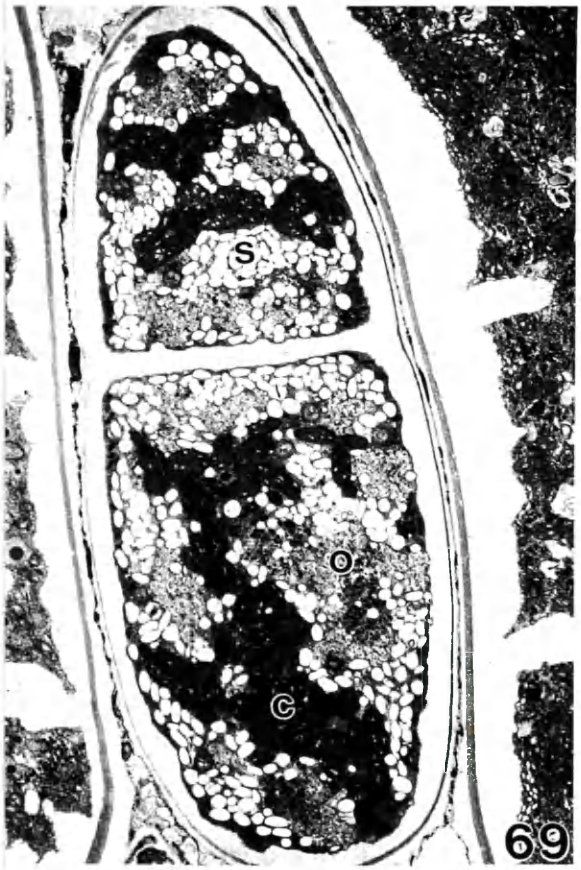
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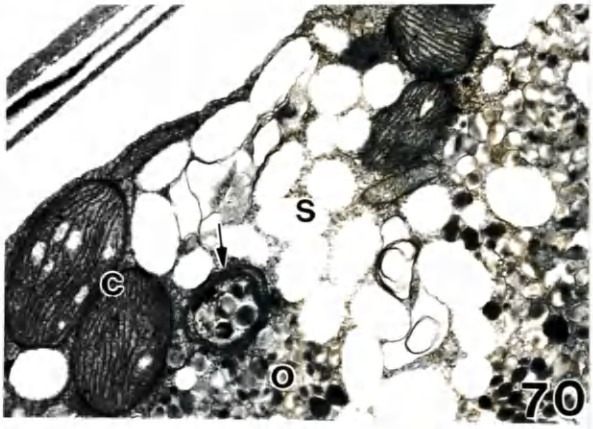
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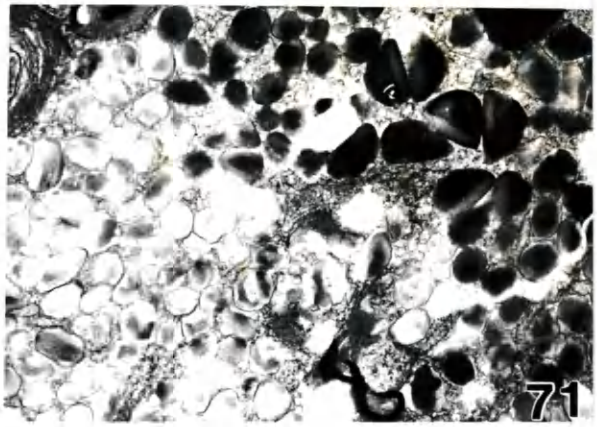
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Figures 72-76. Transmission Electron Micrographs of Stage 3C Bisporangia of *Lithothamnium phymatodeum.*

Figure 72. Bisporangium with irregularly shaped nuclei (asterisk) and dispersed cytoplasmic contents. Irregular cleavage furrow (double arrow) indicates that cleavage has resumed. x 666.

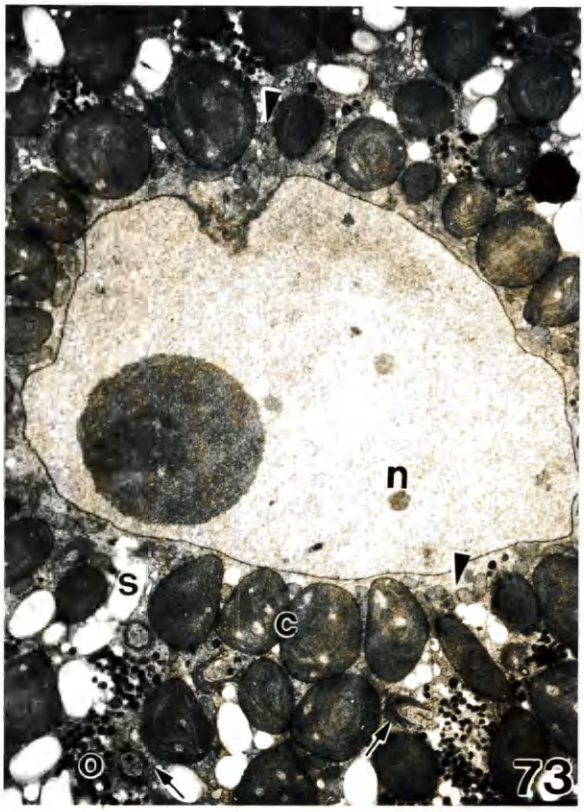
Figure 73. Irregular nucleus (n) with a chloroplasts (c) and mitochondria (arrowheads) association. Starch (s); Golgi (arrows); osmiophillic vesicles (o). x 4,080.

Figure 74. High magnification of cleavage furrow in Figure 72. A track of SER (arrow) connects the furrows. x 5,520

Figure 75. High magnification of dispersed contents of bisporangium with mature chloroplasts (c), starch (s), osmiophillic vesicles (o), small electron-translucent vesicles (asterisks) and lipid globules (L). x 5,520.

Figure 76. SER cisternae (asterisk) associated with cleavage furrow in Figures 72 and 74. x 26,880.


72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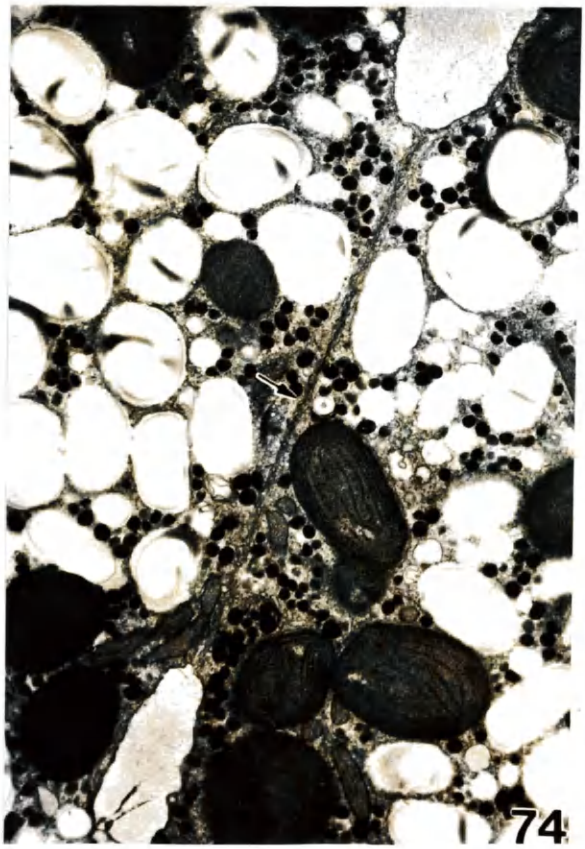
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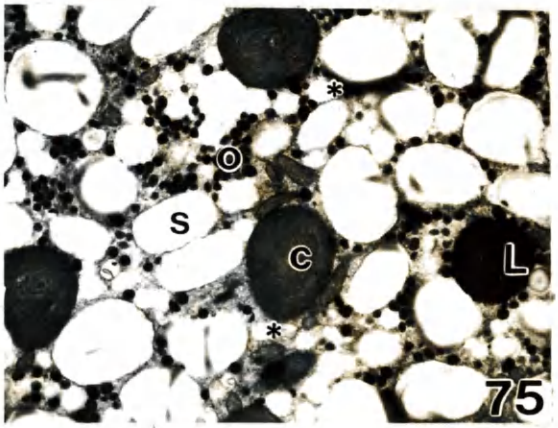
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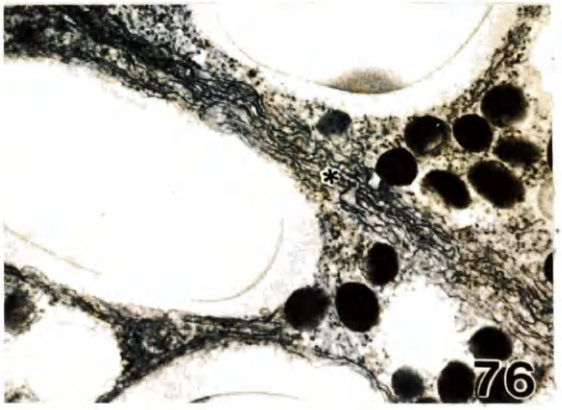

76

Figures 77-79. Transmission Electron Micrographs of Stage 3C Tetrasporangia of *Mesophyllum lamellatum.*

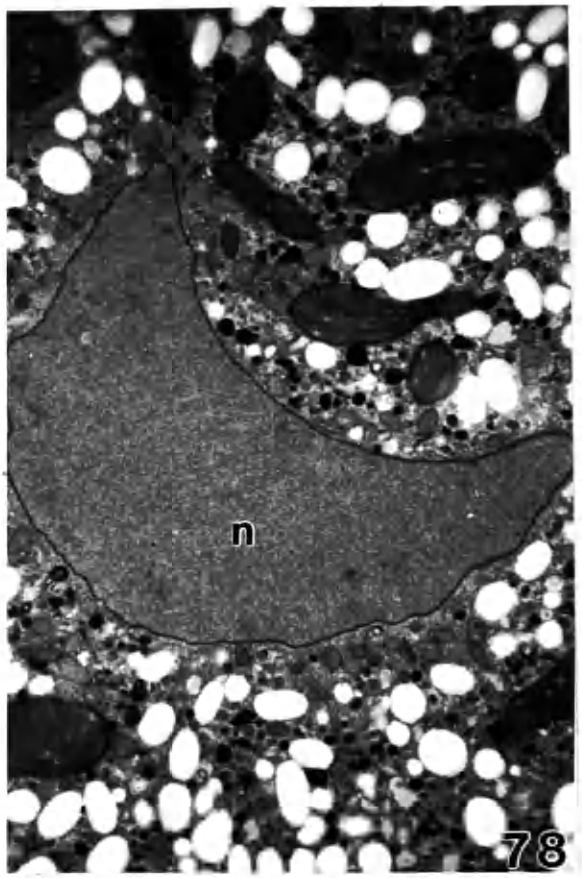
Figure 77. Tetrasporangium with dispersed cytoplasmic contents. x 432.

Figure 78. Irregularly cresent-shaped nucleus (n) lacking any association with organelles. x 6,120.

Figure 79. Higher magnification of dispersed cytoplasmic contents. Starch (s); osmiophillic vesicles (o); mature chloroplasts (c); lipid globules (L); mitochondria (m); small electron-translucent vesicles (asterisk). x 12,960.

Figure 80. Scanning electron micrograph of *Mesophyllum lamellatum*'s owl impression. What a HOOT; this thesis is complete!! x 444.


77



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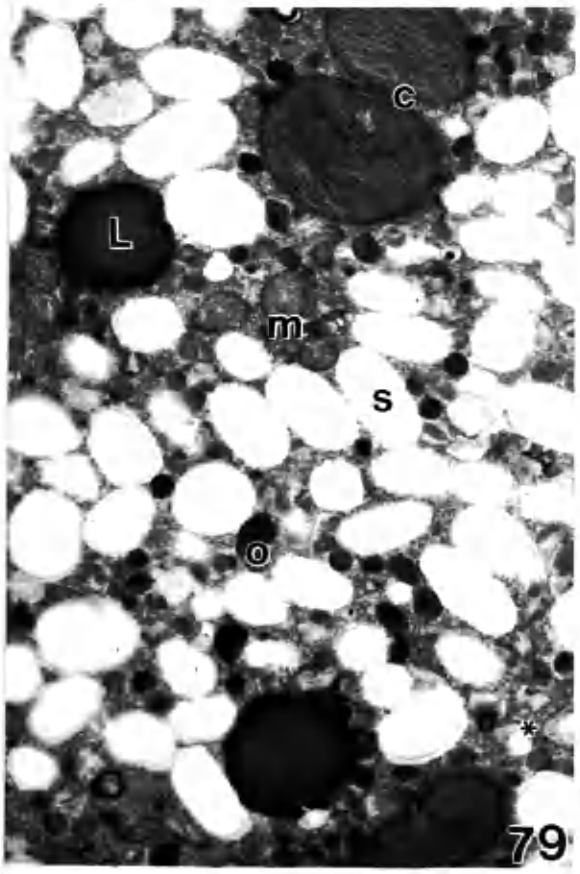

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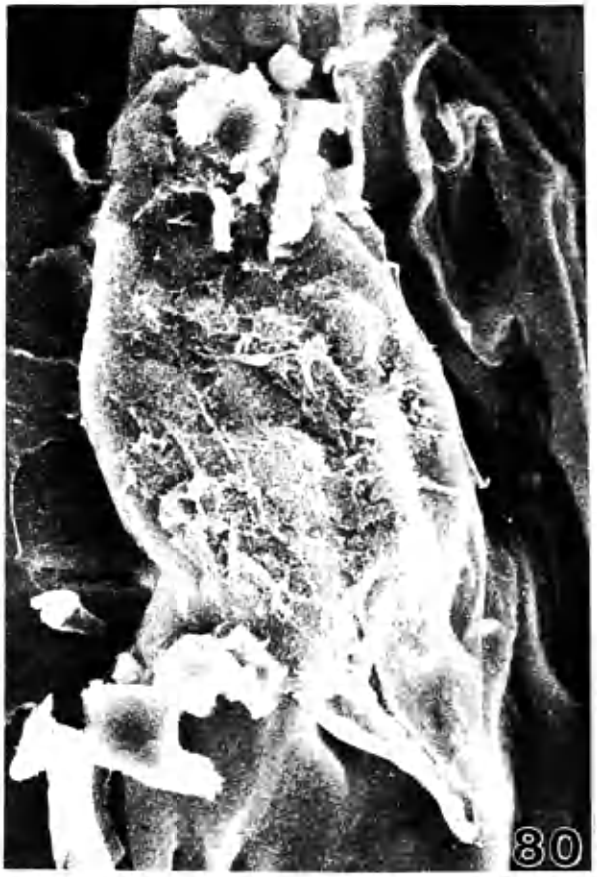
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VITA

Bethany Ann Griffin was born on September 9, 1973 in Greensburg, Pennsylvania. She graduated with high honors from Greensburg Central Catholic High School in May 1991. In May 1995, she graduated cum laude from the Univeristy of Dayton, Dayton, Ohio. In August of 1995, she matriculated into the College of William and Mary's graduate program in the Department of Biology where she is a teaching assistant.