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An Ultra Structural Study of Sporogenesis, Vegetative Morphology, and Host-Parasite Interactions in Choreonema thuretii (Corallinales, Rhodophyta)

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AN ULTRASTRUCTURAL STUDY OF SPOROGENESIS, VEGETATIVE MORPHOLOGY, AND HOST-PARASITE INTERACTIONS IN CHOREONEMA THURETII (CORALLINALES, RHODOPHYTA)

A Thesis Presented to
The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Elizabeth A. LaPointe
1995
APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Arts

Approved, September 1995

[Signatures]

Sharon T. Broadwater
Joseph L. Scott
Mark R. Patterson
DEDICATION

For my husband and best friend, Gregg LaRoche, in acknowledgment of his support and love in our first chapter together.
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Abstract

The monotypic genus *Choreonema thuretii* has not previously been examined at the ultrastructural level. This study utilized transmission and scanning electron microscopy, coupled with transmitted light microscopy and energy dispersive x-ray analysis to survey the vegetative and reproductive features of this red alga and to evaluate its taxonomic position within the Corallinaceae. Ultrastructural details of sporangial development were consistent with those observed in other corallines. No electron dense material (EDM) was found to be associated with the sporangial nuclei. Although no recommendations were made for a change in subfamilial placement, a number of surprising features were observed that corrected previous research, and uncovered new evidence for host-parasite interactions. *Choreonema* was found not to have a conceptacle comprised of a single layer but instead possessed one of several layers with a defined epithallial layer. Most vegetative cells were found to be multinucleate, a feature never before observed in any coralline red alga. Thallus vegetative cells were observed to derive small conjoiner cells that utilize finger-like projections that extend through the host wall and apparently penetrate the cell in a presumptive parasitic interaction.
AN ULTRASTRUCTURAL STUDY OF SPOROGENESIS,
VEGETATIVE MORPHOLOGY,
AND HOST-PARASITE INTERACTIONS IN
CHOREONEMA THURETII (CORALLINALES, RHODOPHYTA)
**Introduction**

The monotypic alga *Choreomena thuretii* (Corallinaceae, Rhodophyta) is a coralline red alga endophytic on three other coralline genera. This investigation uses transmission and scanning electron microscopy coupled with transmitted light microscopy to examine tetrasporogenesis and carposporogenesis in an attempt to further delineate the taxonomic position of this unique alga. Also of interest are details of the relationship between *Choreonema* and its host, whether or not the conceptacles are calcified, and the nature of the *Choreonema’s* vegetative filaments and their role in conceptacle formation.

**Phylum Rhodophyta**

Among the oldest of eukaryotic algae, the phylum Rhodophyta is comprised of a single class, Rhodophyceae, which contains 17 orders (Gabrielson and Garbary, 1986; Bailey and Chapman, submitted). The red algae enjoy a variety of diverse morphologies that include unicells and filamentous and pseudoparenchymenous thalli, some of which are calcified. The main features separating Rhodophyta from other phyla are a lack of centrioles or a flagellar apparatus in any lifestage, possession of pit connections, and floridean starch (α-(1,4)-linked glucan) as a storage product outside of the chloroplasts. In addition, chloroplasts are delimited by two membranes, have unstacked thylakoids, and contain only chlorophyll a, as well as phycobiliproteins. These latter pigments include phycoerythrin (which is responsible for the usually red color of the algae), as well as phycocyanin and allophycocyanin, all of which are located in phycobilisomes, special structures on thylakoids. Zeaxanthin is the dominant carotenoid, although α and β
carotene, and lutein are also present (Brawley and Wetherbee, 1981; Gantt, 1990).

**Order Corallinales**

The Order Corallinales is composed of two families, Sporolithaceae and Corallinaceae. Members of the Corallinales are distinguished from other red algae by the presence of calcite in the walls of vegetative cells. Other important features include pit plugs covered by two-layered dome-shaped caps without cap membranes, reproductive structures maintained within a roofed conceptacle with one or more exit pores, and simultaneous zonate division in tetrasporocytes (Johansen, 1981; Woelkerling, 1988). The family Sporolithaceae is an exception as the two included genera do not utilize roofed conceptacles and tetrasporocytes undergo non-simultaneous cruciate division (Verheij, 1993).

The most notable characteristic of the Corallinaceae is that they are heavily calcified. Calcium deposition is in the form of calcite (the hexagonal-rhombohedral form of CaCO₃); however, skeletons may also contain 7-30% magnesium carbonate. These deposits are formed within the organic matrix of the mucilage and cell walls of the algae (Borowitzka, et al., 1974; Cabioch and Giraud, 1986; Craigie, 1990). The calcified nature of all corallines makes them extremely competitive in areas of high wave intensity (Johansen, 1981; Steneck, 1990). Unlike most groups of red algae, calcification has created an extensive fossil record of corallines that includes over 30 genera (Kraft, 1981). At one time, their calcified nature led the group to be included with coral animals and they were first labeled ‘zoophytes’ by Linnaeus (in Johansen, 1981; Silva and Johansen, 1986). During the 1800’s, they were recognized as members of Rhodophyta and were included as a family within the Gigartinales. When the order Cryptonemiales was created, the corallines were included (Woelkerling, 1988). Cryptonemiales was later subsumed into the Gigartinales due to the lack of consistent morphological differences (Kraft and Robins, 1985). Soon
thereafter, the family Corallinaceae was raised to ordinal status by Silva and Johansen (1986).

Toward the end of the 19th century two main groups of corallines were recognized: the geniculate and the nongeniculate forms. Geniculate corallines have calcified regions called intergenicula which alternate with uncalcified regions known as genicula (Pearse, 1972). Nongeniculate forms do not possess genicula and generally form crusts or mounds over the substrate. It has been proposed that encrusting forms may have evolved to survive in areas of high predation (Steneck, 1990).

The Order Corallinales contains one fossil family, the Solenoporaceae and two extant families (Table 1); Sporolithaceae and Corallinaceae. The Sporolithaceae consists of only 2 genera, Heydrichia and Sporolithon, which are characterized by cruciate tetrasporangia, both secondary pit plugs and cell fusions, and an absence of tetrasporangial conceptacles (Verheij, 1993; Townsend, et al., 1994; Keats and Chamberlin, 1995). Corallinaceae contains at least 36 genera and is delimited by the presence of conceptacles and zonate tetrasporangia which undergo simultaneous cleavage. The recent removal of the family Sporolithaceae from members of the Melobesioideae (Verheij, 1993) has met with agreement from most researchers in the field. The organization of the subfamilies within the Corallinaceae, however, has not met with the same agreement. No less than twelve different schemes have been proposed for the group (Woelkerling, 1988). Most recently, debate has focused upon a scheme devised by Cabioch (1971a, b; 1972; 1988) and supported by Chamberlain (1978) in which the primary character of importance is the type of secondary cell connection as opposed to the more accepted scheme by Johansen (1981) and Woelkerling (1988) who primarily utilize the presence or absence of genicula to define subfamilies. Characters other than secondary cell connections used by both groups to delineate subfamily groupings include the occurrence of apical plugs in bi/tetrasporangia, morphological features of genicula, and the number of pores in the bi/tetrasporangial
conceptacle.

The taxonomic treatment of the Corallinaceae devised by Johansen (1981) and supported by Woelkerling (1988) includes seven subfamilies: three geniculate forms (Amphiroideae, Corallinoideae, and Metagoniolithoideae) and four nongeniculate forms (Melobesioideae, Mastophoroideae, Lithophylloideae and Choreonematoideae). In contrast, Cabioch (1971a; 1972; 1988) has combined geniculate and nongeniculate forms into two of her five subfamilies. Her initial emphasis supported by Chamberlain (1978) is on cell connections (secondary pit connections or cell fusions) to differentiate subfamilies and the presence or absence of genicula is used only at the level of tribe and genus. Her scheme is based on evolutionary concepts more than Johansen and Woelkerling and her organization reflects 1: groups that appeared to branch early, 2: intermediate forms, and 3: groups showing advanced traits (Johansen, 1981).

Although life-histories vary to a certain degree among different genera, members of the Corallinaceae generally show a triphasic life cycle with a haploid gametophyte, a diploid carposporophyte and a diploid tetrasporocyte (Diagram 1). Most are dioecious in nature with separate male and female thalli. Spermatia are produced from spermatangial initials that mature into spermatangia. When spermatia are released, they can be passively carried by currents to conceptacles on the female thallus where they land and fuse (plasmogamy) with the extended trichogyne of the specialized egg cell known as the carpogonium. The spermatial nucleus (or nuclei) migrates down the trichogyne and karyogamy occurs when the two gamete nuclei fuse producing a diploid zygote. The zygote remains within the conceptacle and develops into a microscopic carposporophyte which consists of a fusion cell and gonimoblast filaments. Gonimoblast filaments are produced from the fusion cell and each is comprised of one or more sequentially developing carposporangia, each producing a single carpospore. After release from the conceptacle, carpospores germinate into diploid tetrasporophytes. These thalli produce tetrasporangia containing tetraspore
initials that undergo meiosis to produce haploid tetraspores. When mature, the tetraspores are released and germinate into separate male and female gametophytes (Woelkering, 1988).

Although the sexual life cycle is considered important for maintaining genetic diversity in red algal populations, many groups also utilize asexual means of propagation. The formation of bisporangia is common and resembles tetrasporangial development. Both uninucleate and binucleate bispores have been observed (Woelkering, 1988) although uninucleate bispores are most common. These are produced from mitotic division and are present in a large number of coralline red algae (Guiry, 1990). Much less common are monosporous trisporous. Propagation of fragments and the production of propagules have been recorded for the nongeniculate forms *Fosliella* and *Pneophyllum* (Woelkering, 1988).

Characteristics of red algal sporangia have long been considered taxonomically significant (Guiry, 1978). Critical traits include the presence or absence of mitosis, meiosis and generative mother cells, the number of nuclei in each spore, the quantity and arrangement of spores in the sporangium, the position of the sporangium on the thallus and the role of spores in the general life history of the alga (Guiry, 1978). In addition, the timing and type of cleavage of spores, whether cruciate, tetrahedral or zonate, are considered significant characters. In the corallines (except for members of the Sporolithaceae), spores are zonately cleaved with cleavage occurring simultaneously (Guiry, 1990).

Many aspects of sporogenesis in red algae have been evaluated at the ultrastructural level (Peel *et al.*, 1973; Scott and Dixon, 1973b; Kugrens and West, 1973a, 1974; Tsekos, 1983a, b; Delivopoulos and Kugrens, 1984b; Vesk and Borowitzka, 1984; Sheath *et al.*, 1987; Delivopoulos and Diannelidis, 1990; Wilson, 1993; Agee, 1995; Karnas, 1995). Characters of interest in these investigations include the nature and timing of starch
deposition in developing spores, mucilage and wall formation, nucleolus morphological variations, the presence of various vacuole types, and dictyosome behavior. In addition, preliminary evidence (Wilson, 1993) suggests that perinuclear associations and organization may be useful as taxonomic indicators of relationships within the Corallinaceae.

**Subfamily Choreonematoideae**

Although originally placed in the Mastophoroideae (Johansen, 1969; 1981) and later the Corallinoideae (Cabioch, 1972), *Choreonema* possesses several features that precludes its placement in either group. In 1967, Zinova (in Woelkerling, 1988) placed *Choreonema* in its own subfamily but failed to provide a latin diagnosis. Later, Woelkerling (1987) provided a formal definition and delimited the subfamily based on four traits: the absence of either secondary pit connection or cell fusions, the presence of bi/tetrasporangial apical plugs, the presence of conceptacles comprised of a single outer layer of cells, and spermatangia borne on both the floor and the roof of male conceptacles.

Secondary pit connections are common in many red algal species and consist of a perforation (aperture) in the cell wall between two non-kindred cells that is blocked by a pit plug (Pueschel, 1990). Although primary pit connections (those connections that link daughter cells) are common in *Choreonema*, the species is reported to lack secondary pit connections. Cell fusions (secondary cytoplasmic unions of vegetative cells) are also reported absent from this subfamily making this alga the only coralline species exhibiting neither secondary pit connections nor cell fusions.

The absence of secondary pit connections and cell fusions suggests two divergent phylogenetic evolutionary theories. *Choreonema* may represent a primitive organism that has not yet developed the connections between its neighboring cells as seen in all other coralline groups, or it may be a parasite with a reduced morphology due to secondary loss.
Typically parasites have reduced characteristics such as the presence of proplastids instead of chloroplasts.

**Red Algal Parasites**

About 50 genera (15%) of red algae are parasitic on other organisms (Goff, 1982; Goff and Coleman, 1984). Two main types of parasitism are recognized. Adelphoparasites, which maintain relationships with only closely related algae, always within the same tribe or family, constitute about 80% of the parasitic red algal relationships (Goff, 1982). Examples of these include *Gardneriella tuberifera* growing on *Sarcodiotheca gaudichaudii*, and *Gracilariophila oryzoides* on its host *Gracilaropsis lemaneiformis* (Goff and Zuccarello, 1994). In contrast, alloparsites are not closely related to their hosts which may be more distantly related red algae such as *Choreocolax* growing on *Polysiphonia* (Goff and Coleman, 1984) *Homsella australis* growing on *Gracilaria furcellata* (Goff, 1982) and perhaps the most studied alloparsite, *Harveyella mirabilis* growing on *Odonthalia floccosa* (Goff and Cole, 1975, 1976a,b; Goff, 1976, 1979a, b). Red algae may even invade invertebrate hosts. A caribbean sponge, *Mycale laxissima* has its spongin filaments permeated by two species of algae, *Arcrochaetium spongicolum* (Rhodophyta) and *Ostreobium constrictum* (Chlorophyta). The two algae are filamentous in nature and intertwine their filaments throughout the spongin skeleton of the host (Rutzler, 1985). During this process, there is tearing of the spongin by the invading algae. Another red alga, *Jania adherens*, forms a skeletal support for the sponge *Dysidea janiae* in a dependent symbiosis where the sponge is never found without its red alga counterpart (Rutzler, 1985). Whether either of these associations are truly parasitic in nature has yet to be determined.

Various theories have been presented about the evolution of parasitism in red algae. In 1918, Setchell (Lee, 1989) proposed that adelphoparasites evolved from mutated
tetraspores or carpospores that settled on the parent and germinated. Because the tissues were similar, they were not rejected by the host. Over time, a decrease in size and photosynthetic ability were selected for and speciation of the parasite occurred resulting in an adelphoparasite. Recent work in Goff’s lab supports this theory (Goff and Zuccarello, 1994; Goff, 1995).

Two theories have emerged to explain the evolution of alloparasites which are more distantly related to their hosts. A theory proposed by Sturch in 1926 (Lee, 1989) suggested that organisms that were normally epiphytic evolved into a parasitic lifestyle by first moving through a semiparasitic stage before becoming fully parasitic. Goff, however, suggests that alloparasites are former adelphoparasites that jumped hosts. Molecular studies in her lab (1995) support this theory for those algae investigated.

Johansen (1981) lists three genera of coralline algae that show apparent parasitic lifestyles. These are *Kvaleya, Ezo,* and *Choreonema* and all were listed as parasitic on other corallines. A fourth coralline alga presumed to be parasitic, *Lesueuria* (Woelkerling and Ducker, 1987) is similar to *Kvaleya* and *Ezo* in its production of haustoria that penetrates into host cells. Woelkerling, (1987) however, considers *Choreonema* to be an endophyte and not a parasite since connections to its host have not been established.

Parasitic red algae range in dependence on their host from being fully autotrophic with highly developed pigmented chloroplasts as in *Erythrocytis saccata* (Kugrens and West, 1974) to having reduced chloroplasts (hemiparasitic) as in *Levriniella gardneri* (Kugrens and West, 1973a) to being fully heterotrophic and maintaining only proplastids as in *Harveyella mirabilis* (Goff, 1979b). None, however, have been reported to lack plastids completely (Goff, 1982). Vegetative cells of parasites have been shown to be both uninucleate and multinucleate and most parasites maintain a complex endomembrane system comprised of both rough and smooth endoplasmic reticulum (Goff, 1982).

The red algal parasites that have been studied by Goff and her laboratory have all
been shown to make connections to their host cells via secondary pit connections (Goff and Coleman, 1984; 1985; 1994; Zuccarello and West, 1994). Following secondary pit formation, a copy of the parasite nucleus is transferred into the host cell. With alloparasites, parasitic nuclei may be transferred but neither DNA synthesis nor karyokinesis occurs even though the parasite nucleus is able to survive for a number of weeks and may induce certain cellular responses in the host that contribute to the success of the parasite (Goff and Zuccarello, 1994). Adelphoparasite nuclei, however, undergo DNA synthesis and karyokinesis and appear to transform the host cells such that there is a dedifferentiation of plastids to proplastids, an increase in mitochondria and an accumulation of starch in the cytoplasm as well as growth of parasitized tissue to produce structures directed or partially directed by the parasite (Goff and Zuccarello, 1994).

**Purpose of Study**

The purpose of this study is three-fold. No previous ultrastructural examination of *Choreonema thuretii* has been undertaken. This study will examine both vegetative and reproductive structures. In the first case, primary emphasis will be on the relationship of *Choreonema* to its host *Jania tenella*. A question exists as to whether *Choreonema* is a parasite (Woelkerling, 1988) and if so, the type of parasitism it exhibits. Because *Choreonema* and *Jania* are in the same family, any parasitic relationship with *Choreonema* would normally be considered adelphoparasitism. As an adelphoparasite, it would be unique in neither forming secondary pit connections nor transferring nuclei to its host (Goff and Coleman, 1994). However, the other three coralline parasitic algae, *Ezo* (Adey et al., 1974) *Kvaleya* (Adey and Sperapani, 1971) and *Lesueuria* (Woelkerling and Ducker, 1987) form haustoria that connect to host cells but these relationships have not been examined at the ultrastructural level.

In the second case, the basic vegetative thallus including conceptacle construction
will be examined using consecutive sections. Aspects of vegetative structure is of interest
due to the reduced nature of _Choreonema_. The reported lack of epithallial cells and of a
conceptacular columella warrants confirmation at the TEM level.

In the third case, an evaluation of the ultrastructure of carposporogenesis and
tetrasporogenesis in _Choreonema thuretii_ will be used to assist in the determination of its
taxonomic placement within the Corallinaceae. The taxonomic significance of
developmental patterns and especially nuclear associations has been shown to be of
importance in evaluating relationships within the Corallinaceae (Guiry, 1990; Wilson,
1993; Karnas, 1995). Understanding the patterns of sporogenesis in this organism may be
important to evaluating the Corallinaceae as a whole.
Materials and Methods

**Transmission Electron Microscopy**

Two different collections of *Choreonema thuretii* were made for this study. In September, 1994, specimens of *Choreonema thuretii* attached to its host *Jania tenella* were collected from Indian Rock, Emerald Cove, Santa Catalina Island, California (Fig. 1), at a depth of 3-4 meters. Specimens were field fixed in 3.0% glutaraldehyde in a 0.1 M phosphate buffer with 2.5% EDTA and 0.25 M sucrose at pH 6.8. After the first 30 minutes at ambient temperature, specimens were fixed on ice for an additional 2.5 hours and then rinsed three times with the EDTA-sucrose 0.1 M phosphate buffer. Post-fixation occurred for 2 hours in 1.0% osmium tetroxide in above buffer. Dehydration began with 50% acetone; specimens were then stained with 2% uranyl acetate in a 70% methanol solution for 16 hours at 4 °C. Dehydration continued in ascending order until specimens were in 100% acetone. Material was infiltrated in EM bed 812 (Electron Microscopy Sciences) overnight and then embedded and polymerized in a 70°C oven for 2-3 days.

A second fixation of specimens collected from the University of Southern California’s Wrigley Marine Science Center at Fisherman Cove, Two Harbors, Santa Catalina Island (Longitude 118°, 29.0’, Latitude: 33° 26.73’) was made on July 6, 1995. *Choreonema* on *Jania* was fixed in either 3.0% or 1.5% glutaraldehyde in 0.25 M sucrose, 0.1 M phosphate buffer (pH 6.8) and 2.5% EDTA for 2 hours. They were then rinsed three times for 30 minutes each in rinse phosphate buffer (see above). Post-fixation occurred for 1.5 hours in 1.0% osmium tetroxide in phosphate buffer. Material was rinsed twice in deionized water, stored in rinse buffer for 2.5 hours, and dehydrated in 50% acetone. Material was stored for 11.5 hours in 70% methanol with 2% uranyl acetate under
refrigeration. Dehydration continued until material was in 100% acetone. Specimens were
infiltrated and then embedded in EM bed 812 and allowed to polymerize.

All embedded material was sectioned on a RMC MT 6000X ultramicrotome, picked
up on one-hole grids, stained with Sato’s lead solution and transferred to formvar coated
one-hole grids. Grids were examined on a Zeiss EM 109 transmission electron microscope
operating at 80 Kv and photographed using T-Max 100 film. Only fixations using the
3.0% glutaraldehyde were used for this study.

**Scanning Electron Microscopy**

Dried specimens of *Jania tenella* with intact *Choreonema thuretii* conceptacles were
dehydrated at ambient temperature. Select samples were then mounted using double-sided
sticky tape on aluminum studs and coated with 20 nm of gold-palladium in a Hummer VII
sputter-coater. Specimens were examined on a AMRAY 1810 scanning electron
microscope operating at 20 Kv and photographed using 35 mm Kodak T-Max 100 film.

**Scanning Transmission Electron Microscopy and Energy Dispersive X-ray
Analysis**

In order to determine whether conceptacles of *Choreonema* contain calcium,
samples underwent elemental analysis at Old Dominion University’s Biology Department.
Whole specimens of *Choreonema* on *Jania* were rinsed in deionized water, dried in a 37°
owen and then placed in a desiccator for three days. Specimens were mounted on double
sided carbon tape on a copper base and coated with 80-100 Å of carbon in a SEM
Autocoating Unit E5200 (Polaron Equipment LTD) sputter coater on an E6900 Vacuum
Base. These were then examined on a Jeol JEM-100CX II Scanning Transmission
Electron Microscope equipped with Kevex Delta 1 energy dispersive X-ray detector
(Beryllium window detector) and analyzed using Quantex V software (Kevex Instruments)
by Michael Adam. Analysis was made at 40 kV with a spot size of 4 and a beam width of no more than 0.705312 mm². The analysis was run without standardization, however ZAF Corrections (Z = atomic weight, A = absorbance, F = fluorescence) for all elements were conducted. Three samples of *Jania* and four samples of *Choreonema* were examined.

**Light Microscopy**

Thin sections (200-250 nm) were taken from resin embedded material with a Dupont diamond knife on a RMC MT 6000X ultramicrotome and placed on a glass slide. These were heated and stained for 5-8 seconds with 1.0% Toluidine blue. Sections were then viewed on an Olympus BH-2 photomicroscope and photographed using Ektachrome 100 film. Fixed material (3.0% glutaraldehyde in a buffered solution—see above) were sequentially dehydrated in ethanol (50%-95%) and embedded in H700 Embed Medium (Energy Beem Sciences, Inc.). Material from both the September 1994 fixation and the July 1995 fixation were used. Following polymerization, material was sectioned with glass knives (4.0 μm) on a JB-4 microtome (Sorval, Dupont) and mounted on ethanol cleaned slides. Sections were stained with 0.1% filtered toluidine blue for 5 minutes and examined on an Olympus BH-2 photomicroscope. All material was viewed under Nomarski differential interference contrast microscopy.

Habit photography was conducted on a Wild Photomakroskop M400 with Ektachrome 100 film. Illumination was provided by an Intralux 6000 lamp.
Results

*Choreonema thurettii* grows endophytically within its coralline host *Jania* (Figs 2-9) with the exception of colorless rounded conceptacles on the host surface (Figs 2-8). Conceptacles which are covered by an exterior layer of somewhat hexagonal shaped cells, average 130 \( \mu \text{m} \) in diameter and range in height from 160 \( \mu \text{m} \) to 170 \( \mu \text{m} \) and (Figs 3-5). Spermatangial conceptacles (Fig. 7), although similar in size, may be differentiated from carposporangial (Fig. 6) and tetrasporangial conceptacles (Fig. 8) by the appearance of a beak around the pore aperture (Figs 5, 7). A thallus of *Choreonema* produces a single conceptacle, but a single thallus of *Jania* may act as a host to numerous *Choreonema* thalli and therefore display all three types of conceptacles.

**Basal And Intermediate Cells**

Conceptacles, spores and vegetative filaments appear to develop from 1-2 large cells which I have termed basal cells. These large cells may be over 37 \( \mu \text{m} \) in length and in some sections appear as wide as they are long. They are located within the host thallus at the interface of the conceptacle with the host (Diagram 2) (Figs 8-10). This pattern has been found in tetrasporangial (Figs 8, 9) carposporangial (Fig. 54) and spermatangial conceptacles (not shown). Basal cells are characterized by diffuse cytoplasm with many mitochondria and vacuoles, extensive endoplasmic reticulum (ER) and a few proplastids (Figs 8-11). Concentric cisternae of ER are occasionally seen (Fig. 10) and electron transparent vacuoles containing a reticulated material are frequently scattered throughout the cytoplasm (Fig. 11). The cells are multinucleate (Figs 8, 10) with one to four nuclei present in a single section. Pit connections appear to be typical coralline connections with a
dome-shaped mass of electron dense material on top of another smaller and less dense cap and lack a cap membranes (Figs 8, 18).

Serial sections through a longitudinally cut tetrasporangium and host thallus have revealed a series of pit connections from the basal cells to the vegetative filaments that invade the thallus of Jania as well as to cells I refer to as intermediate cells which are produced in an opposing direction to that of the vegetative filaments (Diagram 2). Intermediate cells are small and numerous but cytoplasmically similar to basal cells (Figs 8-10). Intermediate cells are extremely irregular in shape and may also be multinucleate (Fig. 24). They tend to form the 3-4 layers of cells that lie between the basal cells and the fertile tissues (Figs 9, 10) and form a disk of tissue beneath the conceptacle. Ultimately, this disk is responsible for deriving both the conceptacles (from the disk periphery) and the reproductive cells (from the central disk region).

**Conceptacle Construction**

Conceptacles are comprised of two cell types. The innermost cells are very long (up to 60 μm) and highly vacuolate (Fig 17). The cells follow along the outside of the developing sporangia and may interject filaments between sporangia (Figs 12, 16). They lack a well defined wall and their cytoplasm exists as long meandering threads. The lack of defined wall structure coupled with the cytoplasmic discontinuities makes these cells particularly challenging to follow between sections. Although in a single section it may appear that there are many layers of these cells, there are in fact usually only 2-3. The extra layers are actually components of one cell.

Determination of the overall vegetative morphology of the conceptacle is difficult. An example of the problem is shown in Figs 12-15 in which the innermost vegetative filament along the left side of the conceptacle appears to end at various points. Closer examination of point A shows that the cell is continuous as it is connected to the region of
darker cytoplasm (Fig. 13). This occurs again at points B and C; however closer examination shows that at both of these points, the cell is actually continuous (Figs 14, 15). When conceptacles are cut in a cross-section (Fig. 16) it is possible to see that these vegetative cells are not only long but also relatively wide (up to 33 \( \mu m \) in some cases) and appear in close association with the developing sporangia. If a conceptacle is sectioned through the surrounding wall (before cutting through most of the fertile tissue), the outlines of vegetative filaments are apparent and it is possible to get an idea of the size and the vacuolate nature of these cells (Fig. 17).

Although extremely vacuolate, innermost vegetative cells that form the conceptacle contain numerous mitochondria, lipid vesicles, and extensive ER and may also be multinucleate (Fig. 19). Most innermost cells are produced from the periphery of the disk of intermediate cells; however, innermost cells may also derive other innermost cells as evidenced by pit connections between filaments (Fig. 18). The chief functions of these cells are to provide the pattern for structural support of conceptacle shape, and to produce the outermost layer of cells (Fig. 20).

The outermost cells are characterized by reduced cytoplasmic content and thickened cell walls (Fig. 20). These cells never have pit connections to each other but are always connected via pit connections to the innermost vegetative cells. Consecutive sections show that more than one epithallial cell can be connected to a single innermost cell. The outermost cells vary in size and shape (Figs 3, 4); however most are about 7-9 \( \mu m \) in length. These cells provide structural support and protection to the reproductive tissue.

**Vegetative Filaments Within The Host Thallus**

Aside from the vegetative cells that make up the conceptacle, *Choreonema* also puts forth long vegetative filaments up to 115 \( \mu m \) (Fig. 22) that has been observed to originate from the basal cells (Figs 8, 21, 22). These cells are multinucleate (Figs 22, 23) with as
many as nine nuclei observed in a single cell. Proplastids are also abundant and appear as dark granular bodies with a vacuolate center but no observable thylakoid structure. Mitochondria are numerous as are vacuoles, many containing an electron dense fibrillar material (Fig. 23). ER is extensive and runs parallel along the length of the cell (not shown). The basal cells put forth only one filament extending in a single direction. Although multiple filaments have been observed, these filaments each originate from different conceptacles which may not be visible within a single thin section. These multiple filaments have never been observed to interact or connect in any way. *Choreonema* vegetative filaments are easily differentiated from host tissue. *Jania* cells (Fig. 24) are characterized by extensive chloroplasts with well developed thylakoids. Starch is abundant in these cells (Figs 76, 78) and most have a large central vacuole (Fig. 54).

Vegetative filaments in the host thallus commonly cut off very small cells I call conjoiner cells (Fig. 8, 9, 73). These cells are cytoplasmically diffuse with few organelles and are never multinucleate. Vegetative filaments will cut off anywhere from 1-3 conjoiner cells observed from a single section. These form complex connections between *Choreonema* and *Jania* and will be described in more detail later.

**Sporogenesis Patterns**

Both carposporogenesis and tetrasporogenesis have been divided into three main stages based on the timing of events (meiosis and cytokinesis) occurring in tetrasporogenesis. During carposporogenesis, sporangia undergo a number of developmental features that have been shown to correspond in type and sequence to those occurring in tetrasporogenesis (Agee, 1995; Karnas, 1995) although developing carposporangia do not undergo meiosis and cytokinesis. However, stages used in tetrasporangial development can be applied to carposporangial development.

Stage 1 cells lack completely or else show only early development of a
tetrasporangial wall. Meiosis occurs in tetrasporangia during late stage 1. Stage 2 sporangia are postmeiotic and have begun cleavage. Stage 2 is the longest stage and has been divided into three substages. Stage 2A details production of wall material and mucilage by the dictyosomes or Golgi apparatus (GA). Stage 2B includes production of two different vesicle types; early production is of osmiophilic vesicles while mid-production is of fibrous vesicles, both by the GA. Late stage 2B is also characterized by the production of starch by the ER. The distributions of these vesicles change and randomize during stage 2C as the cell reorganizes itself in preparation for cleavage. Stage 3 spores are post-cleavage and the production of a spore wall and peripheral tubules are seen. The general sporogenesis patterns are summarized in Table 2.

**Tetrasporogenesis**

Tetrasporangial conceptacles are nearly spherical in shape. At the apex of the conceptacle is a single pore (Fig. 5, 7, 26) through which mature spores are released. Each tetrasporangium produces a conical mass (apical pore plug) that appears mucilaginous in content and extends upward to the apex of the conceptacle (Figs 25, 27). These pore plugs collectively block the pore of the conceptacle. Tetrasporangia arise from and are pit connected to stalk cells (Figs 28-30). Stalk cells lack a discernable cell wall and have a dense, fairly homogenous cytoplasm with a small number of organelles and a few small vacuoles. A single large nucleus is distinguished by a prominent nucleolus and a nuclear envelope perforated by a large number of nuclear pores (Fig. 30). Stalk cells arise from intermediate cells that may be irregularly shaped and multinucleate (Fig 28). These cells may have 2-4 nuclei which are very similar in size and appearance. Pit connections from the stalk cells though 2-4 intermediate cells eventually conclude at the large basal cell (Diagram. 2)

**Stage 1-Pre-Meiosis/Meiosis**
Early pre-meiotic tetrasporangia are irregularly shaped, elongate cells (around 8.0 μm in length and 3.0 μm wide) and most commonly found toward the center of the sporangial conceptacle (Figs 31-33). The cytoplasm is dense with a large central nucleus and numerous vacuoles (Fig. 32). The GA, which is always associated with mitochondria, exhibits a straight profile and appears largely inactive (Fig. 34). Proplastids are observed in early stages, however the density of the cytoplasm makes them difficult to differentiate from mitochondria (not shown). They appear in the same form as those found in the vegetative cells of *Choreonema* (Fig. 23). Although few vesicles are produced at this stage, large lipid droplets (0.5 μm diameter) are occasionally seen and maintained through all stages of maturation (Fig. 34). Long strands of ER traverse the cell (Figs 33, 34) but do not appear to be associated with the nucleus. The nuclear envelope is perforated by numerous pores and the nucleus contains a dark staining nucleolus (Figs 31, 33, 37) that is frequently asymmetrically located within the nucleus and very closely associated with the nuclear envelope. A tetrasporangial wall may be absent in very immature spores; however, early production of wall material is sometimes apparent (Fig. 35). Grainy vesicles (0.2-0.3 μm diameter) appear in late stage 1 and are thought to be produced by the ER. These vesicles are characterized by a spherical shape with a granular appearance (Fig. 36). The ER often appears associated with the periphery of the cell (Fig. 35).

Late in stage 1, meiosis occurs and 4 haploid nuclei are produced. Because meiosis is a rapid event, it is rare to find cells in this state. Only one cell was found in any stage of meiosis. In the one case, the cell contained a nucleus in zygotene of prophase I as determined by the presence of synaptonemal complexes observed bisecting the condensed chromatin in the nucleus (Fig. 37).

**Stage 2-Post-Meiosis/Cleavage**

Substage 2A tetrasporangia show increased wall development as the GA produces electron-translucent material that is exocytosed (Figs 38, 39). This activity gives the wall
layers an irregular appearance (Fig. 38). Golgi cisternae appear dark and oppressed, at times seen as a dark smear outlining a corner of a mucilage vacuole (Fig. 39). ER is present along the periphery of the cell and near but not yet associated with the nucleus (Fig. 38, 39). Partial cleavage of the cell occurs during this stage but is arrested by the subsequent stage (Figs 38, 40). The cleavage furrows extend deeply into the cell leaving a core of continuous cytoplasm that is approximately one third the diameter of the cell (Fig. 40). Cytokinesis remains in arrest until very late stage 2 (Fig. 48). Grainy vesicles are still present throughout the cell (Figs 38, 39).

During early substage 2B, the developing tetrasporangia exhibit a loss of vacuoles, and a solidified mucilage layer appears inside the tetrasporangial wall (Fig. 40). The production of osmiophilic vesicles by the GA, which in early stages may have a more electron-transparent periphery, occurs in early substage 2b (Figs 40-42). These vesicles are about half the size of the grainy vesicles (0.15 μm diameter), occur in small clusters throughout the cell and are still present in stage 3 tetraspores. The GA show a curved profile during osmiophilic vesicle production (Fig. 42). During this stage, the ER may begin association with the nucleus (Fig. 41); the interaction, however, is not yet close enough to be considered perinuclear ER (PER). At intervals around the nucleus, five or more tracks of ER may be present which then veer off into the cytoplasm (Fig. 41).

Tetrasporangia in late substage 2B are characterized by the presence of starch and fibrous vesicles produced by the GA (Fig. 44). Osmiophilic vesicles are present but more randomly scattered throughout the cytoplasm; however, grainy vesicles have disappeared (Figs 44-47). During production of fibrous vesicles, GA becomes swollen with curved profiles (Figs 45, 47). Fibrous vesicles (0.2-0.4 μm diameter) when first produced are found clumped together and eventually become the most numerous organelles within developing tetrasporangia (Fig. 44). During fibrous vesicle production, starch first appears lying parallel to the tracks of ER. The ER tracks next to the nucleus extend into the
cytoplasm and appear to compartmentalize the cell (Fig. 46). The spokes of ER extend out toward the plasma membrane and then run parallel to the cell periphery (Fig. 47). Both starch and fibrous vesicle production continue throughout this stage (Figs 46, 47).

Substage 2C is characterized by a randomization of all vesicle types (Fig. 48). Starch disassociates with the ER which loses its spoke-like appearance but remains near the nucleus and cell periphery (Figs 49, 50). Proplastids become more obvious at this stage as they show up clearly against the background of fibrous vesicles (Fig. 50). Proplastids appear as small organelles with a vacuolate center and are frequently seen in clumps near the nuclei.

**Stage 3-Post-Cytokinesis**

Stage 3 tetrasporangia are characterized by cleavage furrows that extend entirely across the cell subdividing it into four distinct tetraspores (Fig. 51). There is a marked decrease in the number of GA, although those that are present show a straight profile and appear inactive. Osmiophilic vesicles, starch and fibrous vesicles are randomly distributed throughout the cytoplasm and the lipid filled droplets described in stage 1 are still present (Fig. 52). Proplastids are present but do not appear to have matured or developed any structures during the maturation of the tetrasporangia. They still closely resemble those found in vegetative cells. Cleavage furrows show a reduction in thickness and their outlines along the length of the furrow may exhibit an irregular shape with adhesions between the two sides of the furrow (Figs 51, 52). Peripheral tubules which are present only after cytokinesis, protrude at right angles into the cytoplasm from the cleavage furrows and along the sides of the plasmalemma (Figs 52, 53). These tubules range in length from 30-32 nm and are about 10 nm in width. They may have some association with or be bordered by ER that is also present along the periphery of the sporangia (Fig. 52). Formation of a tetraspore wall (arrowheads) takes place outside the plasmalemma but inside the tetrasporangial wall and mucilage layer (Figs 52, 53).
Carposporogenesis

The carposporangial conceptacle (Fig. 54) is very similar in shape and size to the tetrasporangial conceptacle. At the base of the conceptacle is a multinucleate fusion cell (Figs 54-57). Fusion cells which are formed after karyogamy in the carpogonium, and transmission of the 2n zygote nucleus to an auxiliary cell, result from a merging of intermediate and auxiliary cells. In the conceptacles observed, the fusion cell is connected to at least one layer of intermediate cells which are in turn connected to the basal cells (Figs 54, 55). Occasionally, remnants of the degenerating carpogonium may still be found in the center of a conceptacle (Fig. 6).

Within the carposporangial conceptacle, the fusion cell produces numerous gonimoblast filaments that are 5-7 cells in length (Fig. 57). The cells are sequentially developing carposporangia with the one most proximal to the fusion cell being the least mature. Carposporangia consecutively show increasing stages of maturity toward the apex of the conceptacle with those found most distal from the fusion cell being the most mature (Fig. 54). The first gonimoblast cell is the dividing cell that produces the developing carposporangia (Figs 58-60). This cell most likely does not differentiate into a carposporangium. Cleavage in these proximal cells is preceded by a line of electron transparent vesicles in the plane of the cleavage furrow (Figs 59, 60).

At the TEM level, the fusion cell has many vacuoles (Figs 55, 56) and numerous mitochondria scattered throughout the cytoplasm. Large vesicles (2.0 μm) contain electron-dense material and appear surrounded by a double membrane and a wall-like material. Alongside these vesicles are electron-transparent vacuoles that contain a darkly staining reticulated material (Fig. 56). ER is present throughout the cell, surrounding the nuclei and following the periphery of the plasmalemma (not shown). The fusion cell may contain more than six nuclei in a single section that are characterized by dispersed
chromatin (Fig. 55). The GA displays a straight profile and do not appear to be active (not shown).

**Stage 1—Early development**

Stage 1 carposporangia, which are developmentally equivalent to stage 1 tetrasporangia, are found in the basal region of the gonimoblast filaments near the base of the carpospore conceptacle. These cells are square to rectangular in shape and lack a noticeable cell wall (Fig. 61). The densely staining cytoplasm has some medium-sized electron transparent vacuoles and numerous mitochondria. The nucleus has darkly staining nuclear pores but does not show any perinuclear associations (Fig. 61) although ER is present in the cell. Large lipid filled droplets are not numerous, but generally found in the cytoplasm at this stage (Fig. 61). These are seen to persist in mature carpospores (not shown). Unlike stage 1 tetrasporangia, carposporangia are slower in their wall development and obvious secretions to this layer are not seen until stage 2. Meiosis does not occur in carposporangia.

**Stage 2—Wall And Vesicle Formation**

Stage 2A cells are easily recognized by the uneven appearance of the cell periphery (Fig. 62). This appearance is due to exocytosis of mucilage produced by the GA (Fig. 65). The GA does not show the oppressed and darkly staining profile seen in the tetrasporangia; however, wall production is begun earlier in tetrasporogenesis. Grainy vesicles are abundant in the cytoplasm (Figs 62, 65).

Early substage 2B is marked by production of osmiophilic vesicles by the GA in various clustered areas (Figs 63, 66). ER is found in tracks of several cisternae which show no apparent association with any organelles and may be found in random orientation (Fig. 63).

Late substage 2B is denoted by the production of fibrous vesicles by the GA (Figs
During this period, the distribution of the osmiophilic vesicles begins to randomize in the cytoplasm (Fig. 64). Concurrent with fibrous vesicle production, starch is produced along the tracks of ER (Figs 64, 68). These ER tracks are found associated with the nucleus and then extend out in the cytoplasm toward the periphery of the cell. ER is also found lying parallel to the plasmalemma. Grainy vesicles are no longer present. Stage 2C is marked by randomization of all vesicle types within the cytoplasm (not shown).

**Stage 3--Maturation**

Stage 3 spores do not undergo cleavage as seen in tetraspores; however, the cellular components of the two sporogenesis patterns remains the same. Stage 3 cells have vesicles with random distributions. Starch is no longer arranged along the ER tracks and these appear less distinct in the cytoplasm (Figs 69, 70). The nucleus remains surrounded by ER, although the spokes that appeared to compartmentalize the cell (Fig. 69) are no longer obvious. The GA are less numerous, and where apparent, have a straight profile and are associated with mitochondria (not shown). Proplastids and lipid droplets are also present (Fig. 70) but fibrous and osmiophilic vesicles take up most of the cytoplasmic space (Fig. 71). The most distinguishing characteristic for mature carposporangia is the presence of peripheral tubules and the formation of a carpospore wall inside the carposporangial wall. Peripheral tubules are present perpendicular to the cell membrane and are oriented at right angles into the cell (Figs 71, 72). These appear to associate with the ER that runs along the periphery of the cell. On the outer edge of the peripheral tubules, the development of the carpospore wall may be seen more clearly than in tetrasporogenesis (Figs 71, 72).

**Choreonema - Jania Interactions**

The long filamentous vegetative cells of *Choreonema* that are found within the host thallus frequently give rise to a number of comparatively smaller cells (1.8 μm) (Fig.
73) that I call conjoiner cells. These, unlike the parent cell that they are pit connected to, are uninucleate and have few organelles within their homogenous-appearing cytoplasm (Fig. 74). A few mitochondria are usually present and a few electron-transparent vacuoles. Proplastids have not been observed in these cells, although a few short filaments of ER may be present. Conjoiner cells are juxatabposed and or connected to host cells (Figs 73, 75, 76). These cells extend tubular-like protrusions that connect with the Jania cells (Figs 74-85). There may be more than one extension toward a host cell (as many as five have been observed) from one conjoiner cell (Figs 77,78) and one conjoiner cell may attach to more than one Jania cell (Fig. 76). Numerous Choreonema cells may also attach to a single Jania cell (Fig. 78) and up to three attached cells have been observed (not shown).

The cytoplasm of Choreonema near the connecting area is distinguished by abundant reticulated vacuoles (Figs 76, 79, 83). The protrusions that attach Choreonema to Jania appear to be tubular-like extensions of Choreonema plasma membrane that project through the cell wall and connect with the plasma membrane of the host (Figs 80, 81, 83, 85). These projections are irregular in shape and meander slightly in their overall path toward the host cells (Figs 80-85). Contents of these extensions may be electron-transparent (Figs 80-82) or electron-dense (Figs 84, 85). The finger-like projections (0.47 \( \mu m \) length) penetrate the cell wall and go into the host membrane (Figs 81, 84, 85). It is extremely difficult to determine the amount of penetration into the host; however, some protrusions appear to show entry into the cytoplasm or possibly fusion with the cell membrane of the host (Figs 81, 82). Older connections (electron-dense) appear to disrupt the inner leaflet of the host plasmalemma. This takes on the appearance of “bubbles” or a sloughing off of materials along the inside of the host membrane (Figs 83-86). This interaction extends down the whole length of the plasma membrane and is not isolated to only the region of connection (not shown). Electron-dense material may be observed in the conjoiner cells putting forth dark extensions (Figs 84, 85). This material then seems to be
injected into host cells (Fig. 84)

**Energy Dispersive X-Ray Analysis (EDX)**

Data from EDX analysis was graphed with the X axis equaling the KeV range of the elements tested (0.00-10.23 KeV) and an arbitrary Y axis that corresponds to a generalized scale based on weight percent of all elements found normalized to 100. Figs 87-93 detail the results of EDX analysis for each of the seven runs tested. Coral. 1-3 correspond to *Jania* (Figs. 87-89) while Nodule’s 1-4 correspond to *Choreonema* (Figs 90-93). Elements represented by copper, iron, chloride and aluminum represent readings from the stub and adhesive.

The results from the EDX data clearly show that conceptacles of *Choreonema* are calcified. On average, conceptacles contained about 62.53 % calcium by weight. Although less calcified than *Jania* (89.39 % by weight) *Choreonema* conceptacles were also high in sulfur (16.72 % by weight) likely due to sulfated polysaccharides in the sporangial mucilage.
Discussion

*Choreonema thuretii* is the sole member of the sub-family Choreonematoideae and is unique among the corallines in possessing neither cell fusions nor secondary pit connections. This study is the first electron microscopic investigation of *Choreonema*. Aspects of its biology addressed include vegetative ultrastructure, conceptacle formation, patterns of sporogenesis, and host interactions. This study also has uncovered a number of traits exclusive to *Choreonema*.

Thallus Construction

After a spore of *Choreonema* lands on a host organism, germination occurs via a germination tube (Cabioch, 1972; Woelkerling, 1987; Zuccarello and West, 1994). Germination may be dependent on the presence of a lesion or other break in the outer cell layers. Goff and Cole (1976a) were unable to germinate *Harveyella mirabilis* on *Odonthalia floccosa* without the presence of a lesion most usually caused by grazing isopods. Other parasites enter host tissue through open pores of reproductive tissue. A study of spore germination in *Choreonema* was not conducted in this study. However, Cabioch (in Johansen, 1981) determined that *Choreonema* spore germination begins with the extrusion of simple germination tubes that grow in a bipolar manner in what has been called the *Naccaria* type of germination. This differs from germination in most other corallines (including *Jania*) which follows a *Dumontia* type. The *Dumontia* type of germination falls into three categories; however all begin with a discoid spore that divides into four cruciately arranged cells. In contrast, the *Naccaria* type of germination begins with the formation of a filamentous protonema. *Choreonema* is not the only coralline algae with this type of spore development; *Amphiroa verruculosa* and *Amphiroa rigida* also
have a *Naccaria* type of germination.

Woelkerling (1987) suggests that the germination tube (i.e., a rhizoid) in *Choreonema* develops into a bipolar vegetative cell. One side of this cell produces a filament and the other side produces fertile tissues. This reasoning appears to match our observations of vegetative filaments and conceptacle formation in *Choreonema*. One of two large basal cells found at the base of all conceptacles may be the remnant of the bipolar vegetative cell. These cells produce an extremely long filament that extends down the host thallus in only one direction. They also produce in the opposing position a number of intermediate cells that derive either the vegetative cells that make up the conceptacle, or the initials that eventually form the reproductive cells.

*Choreonema* exhibits a *Polysiphonia* type life history meaning that all life history stages look the same except for the beaked roof of the spermatangial conceptacle. Except for the conceptacles, the thallus is completely endophytic and so reduced that the use of many of the terms in the literature used to describe the different cell layers is inappropriate for this organism. Adey and Sperapani (1971) suggested that the elongate vegetative cells embedded in the host thallus in *Choreonema* are homologous to a hypothallium (usually described as the lower cells in a thallus). They then assumed that the small cells bordering hypothallial cells must then be homologous to epithallial cells (terminal cells that make up the outside layer) embedded in the host. Although Woelkerling (1988) did not address the issue with respect to *Choreonema*, he has rejected the terms hypothallium and perithallium (defined as cells occupying the upper part of the thallus) using instead primigenious and postigenous. Primigenious cells originate from the spore and form a single layer of cells from which arise postigenous cells at right angles. Although *Choreonema* does not produce the multi-layers of cells for which these words define, the placement within the thallus of basal cells, long vegetative cells and intermediate cells suggests that basal cells and vegetative filaments may be homologous to primigenious cells while the intermediate
cells may be homologous to postigenous cells. Regardless, I decided to use simple positional terms with the exception of conjoiner cells which is a new name based on conjunctor cells, the small cells used in some red algal species during secondary pit connection formation. It is certainly possible that conjoiner cells represent reduced epithallial cells as suggested by Adey and Sperapani (1971). Their contention is given greater strength in this study by finding epithallial cells in the conceptacle (discussed later).

Vegetative cells of *Choreonema* include the large primigenous basal cells located beneath the conceptacle, long filamentous cells that extend down the host thallus and produce the small conjoiner cells, intermediate cells that are found between the basal cells and the fertile tissues, and two types of conceptacle cells: innermost and outermost cells. All cell types appear to originate from the basal cells which may in fact be meristematic in their ability to form different cells types. However, this ability may last only as long as the reproductive conceptacles are present. Basal cells produce a number of irregular nonpalisade intermediate cells that are pseudoparenchymatous in nature (Woelkerling, 1988). Intermediate cells then produce the vegetative cells that ultimately construct the conceptacle (innermost cells) and the outside layer (outermost cells). Inside the host thallus, basal cells produce long vegetative multinucleate filaments. These filaments have been observed to possess neither cell fusions nor secondary pit connections and do not form connections between filaments from other conceptacles. Although observations that these filamentous cells are branched appear in the literature (Woelkerling, 1988) observations at the TEM level in this study revealed no thalli with this configuration. The vegetative cells produce several small lenticular-shaped cells that I call conjoiner cells but these cells will be discussed later.

**Conceptacles**

Except for the Hildenbrandiales, only coralline red algae have reproductive tissues
housed within conceptacles (Johansen, 1981). However, the conceptacles of the Hildenbrandiales have a very different developmental sequence and should not be considered homologous structures (Pueschel and Cole, 1982). Taxonomically, conceptacles are important both to separate corallines from other groups of red algae and to help distinguish between subfamilies in the Corallinaceae (Johansen, 1972).

The diagnostic description of *Choreonema thuretii* (Woelkerling, 1987) indicates that the conceptacle is only one cell layer in thickness and lacks epithallial cells. Contrary to Woelkerling's findings, this study shows at least two types of cells comprising the conceptacles; an innermost layer of wall-less vegetative cells and an outermost layer with a thick cell wall and reduced cytoplasm. Examination particularly of innermost layers is difficult even with transmission electron microscopy. The difficulty lies in the extreme vacuolate nature of the innermost cells. Coupled with a thread-like cytoplasm that differs in density and an irregular arrangement of cytoplasmic filaments, it is challenging to determine the number and actual size of these cells. However, pit connections between the innermost layer and the outermost layer are obvious in numerous micrographs indicating that the innermost cells produce the outer layer.

In *Choreonema*, intermediate cells within the central region produce reproductive initials while those on the periphery remain sterile and produce the innermost cells that construct the rounded conceptacle. Innermost cells may be multinucleate and form multiple layers; however, no more than three layers of cells have been seen and then only near the base of the conceptacle. Innermost cells occurring closest to the developing sporangia are the longest and may extend to the apex of the conceptacle. Those further away tend to produce outer cells near the middle of the conceptacle. The shortest innermost cell always occurs near the base of the conceptacle closest to the conceptacle wall. Here, it produces the first outermost cell closest to the host tissue.

The innermost layer of cells produce the thickened outermost cells that cover the
conceptacle. Outermost cells are uninucleate and much smaller in dimension than the innermost cells. Primary pit connections with domed caps typical of coralline algae are found between the innermost and the outermost cells. However, pit connections were never observed between any contiguous outermost cells. In his description, Woelkerling (1987) reported that the conceptacle of *Choreonema* was only one cell layer in thickness and lacked true epithallial cells. Contrary to Woelkerling's findings, this outer layer of cells appears to be an epithallial layer. However, it is almost certain that innermost cells can produce several monostratose epithallial cells at the same time. Such construction has not been reported before in the literature and may warrant a re-evaluation of the terminology.

Epithallial cells of *Choreonema* are calcified, but apparently as is the case in *Jania*, the upper surface of these cells is uncalcified and collapses down onto the calcified lower portions during SEM preparation (Johansen, 1981). In appearance, at least, the epithallial cells of *Choreonema* look like those of its host which give the exterior of *Jania* an appearance that is distinctive and designated a *Jania* type thallus (Garbary and Johansen, 1982). *Jania* type thalli are found only in the tribe Janieae (which includes two other hosts to *Choreonema, Cheilosporum* and *Haliptilon*) and superficially in *Clathromorphum* (Garbary and Johansen, 1982). The close resemblance between the surface of *Jania* and the conceptacle surface of *Choreonema* led Carrillo et al., (1986) to propose that *Choreonema* represents an evolutionary regressive tribe of the Corallinoideae.

Multinucleate Cells

Although numerous species of algae exhibit multinucleate cells, the evolutionary trend is that cells have a single nonpolygenomic nucleus (Goff and Coleman, 1990). Those algal species that exhibit multi-nucleate cells include members of the Gigartinales, Palmariales, Rhodymeniales, Ceramiales, Delesseriaceae, Dasyaceae and the
Rhodomelaceae (Goff and Coleman, 1990). Until now, no members of the Corallinaceae have been shown to possess multinucleate vegetative cells, except in those genera where cell fusions occur and the nuclei appear free to enter other cells (Johansen, 1981). Observations of multinucleate cells at the base of polysporangia have been made in *(Tiffaniella) Spermothamnion snyderae* (Drew, 1937) and in *Pleonosporium vancouverianum* (Sheath, *et al.*, 1987), both being in the order Ceramiales. Although polysporogenesis is considered to be homologous to tetrasporogenesis, a main difference is that polysporangial initials are multinucleate while tetrasporangial initials (or stalk cells) are uninucleate. Although it is the multinucleate vegetative cells that ultimately produce the polysporangia, it is unlikely that this condition in *Choreonema* is linked. Stalk cells observed in *Choreonema* are uninucleate although they may be produced from multinucleate intermediate cells.

It has been noted that most Florideophyceae maintain a constant nuclear volume to cell volume (Coomans and Hommersand, 1990; Goff and Coleman, 1990). The production of multinucleate cells occurs when nuclear division takes place without cell division. This increase in nuclear volume would then be accompanied by an increase in cellular volume (Coomans and Hommersand, 1990). Vegetative filaments in *Choreonema* are extremely long; one observation of 115 $\mu$m filament possessed nine or more nuclei. Basal cells are also very large. The extreme sizes in *Choreonema* cells may be a result of maintenance of cellular and nuclear volume ratios.

**Sporogenesis**

Patterns of sporogenesis are considered to be potential indicators of phylogenetic relationships (Kugrens and West, 1974; Guiry, 1990). All sporangia undergo extensive cell reorganization that includes differential timing and production of various cellular vesicles, changes in the distribution and number of organelles including mitochondria,
plastids, ER and GA and production of protective and or adhesive walls upon maturation. Spores, during maturation, go from being densely cytoplasmic, wall-less and organelle poor to having extensive production of different types of vesicles as well as development of a sporangial wall. As in most corallines, *Choreonema* tetrastcorporangia cleave in a simultaneous zonate pattern that produces four haploid spores. Pre-released tetra- and carpospores are also characterized by the production of a spore wall (Vesk and Borowitzka, 1984).

Sporogenesis in *Choreonema* begins with the production of stage 1 spores. These spores lack detectable cell walls, have inactive GA and have electron transparent vesicles in their cytoplasm. Grainy vesicles are produced by the ER near the end of stage 1. Meiosis and the production of cleavage furrows ends stage 1 in tetrasporangia. Stage 2A is characterized by the GA producing electron-translucent vesicles thought to contain mucilage for the mucilage layer inside the developing sporangial wall. The GA then produces osmiophilic vesicles during early stage 2B, and these remain in a clumped distribution until late stage 2B which is characterized by the production of fibrous vesicles. Concurrent with fibrous vesicle production, starch forms along the cisternae of ER. Stage 2C exhibits randomization of all vesicles in the cytoplasm and a disappearance of grainy vesicles. Cleavage is complete by stage 3 which can be recognized by the presence of peripheral tubules perpendicular to the plasmalemma. Outside this membrane, a spore wall develops.

Patterns of sporogenesis in *Choreonema* carposporangia and tetrasporangia match the observations of Agee (1995) and Karnas (1995) in which both carposporogenesis and tetrasporogenesis appear to undergo similar development. Thus studies of sporogenesis of one part of a life cycle may be correlated to similar events in another. Aside from meiosis and cytokinesis, the only major difference between tetrasporogenesis and carposporogenesis in *Choreonema* is the timing of sporangial wall formation. In tetrasporogenesis, early exocytosis of vacuoles and wall material may be observed prior to
meiosis (stage 1) whereas wall development in carposporangia begins in stage 2. This difference may be due to development of cleavage furrows seen in early stage 2 following meiosis.

Aspects of cystocarp development have been considered taxonomically important in the corallines. The family Corallinaceae was placed for a while in the order Cryptonemiales based on studies of carposporangial conceptacles (Lebednik, 1977). Important aspects of cystocarp development include the position and function of auxiliary cells (Tsekos, 1983a), the type of fusion cell that develops (whether continuous as in *Choreonema*, discontinuous as in *Lithophyllum*, or broken as in *Melobesia*.) (Delivopoulos and Kugrens, 1984a; Delivopoulos and Tsekos, 1985; Delivopoulos, 1990) and the position of developing carposporangia initiation along the fusion cell.

Fusion cells have been considered to serve as a nutritive function during maturation of carposporangia (Hommersand and Fredericq, 1990). Some fusion cells in *Choreonema* possess numerous large vacuoles that contain unidentified materials. These may serve a nutritive function for developing carposporangia as sterile nutritive cells are absent as are starch reserves. Both gonimoblast cells and vegetative filaments are found pit-connected to the fusion cell. The vegetative filaments are the cells responsible for the structure of the conceptacle and are pit connected to the epithallial layer.

Early development of tetrasporangia follows the general patterns observed in most other corallines. Bisporogenesis is said to occur in *Choreonema*, however, no observations of this spore pattern were found. Stage 1 tetrasporangia are formed from a stalk cell. Pit connections between stalk cells and mature sporangia can often be observed. Unlike *Palmaria palmata* where a cell wall forms around the stalk cell before it cuts off a stage 1 tetraspore (Pueschel, 1979), the formation of a distinct cell wall does not occur in stalk cells and only occurs in the sporangia toward the end of stage 1.

During late stage 1, meiosis occurs and produces four haploid nuclei. Because
meiosis is a rapid event, only one observation of a meiotic stage 1 tetrasporangium was made. The cell appeared to be in prophase I, as indicated by the presence of synaptonemal complexes (Kugrens and West, 1972). Few studies of meiosis have been attempted in the red algae. A study of meiosis in *Dasya baillouviana* (Broadwater *et al.*, 1986a; 1986b) has shown that meiotic prophase I is very different than mitotic prophase. A main difference is the appearance of synaptonemal complexes that work to pair corresponding regions of homologous chromosomes. These usually disassociate before prophase I ends. In *Dasya*, polycomplexes (polymerized units of synaptonemal complexes) were maintained in postmeiotic cells. No observations of polycomplexes have been seen in postmeiotic cells of *Choreonema*.

Because developmental patterns of tetrasporogenesis and carposporogenesis are the same, the following discussion refers to both unless otherwise stated.

**Nuclear Associations**

In the first study of tetrasporogenesis in a coralline red alga, Peel *et al.*, (1973) noted an electron dense material (EDM) around the tetrasporangial nuclei. Wilson (1993) suggested that the presence or absence as well as the arrangement of EDM could be used as a taxonomic character that could help to define the subfamilies within the corallinales. She described two types, the *Corallina* type which resembles large eyelashes around the nuclei in later stages and the *Bossiella* type which remain more closely oppressed to the nuclear envelope. *Corallina* type EDM begins with EDM filled invaginations of the nuclear envelope that go from a tight association to a more diffuse association. In addition to *Jania* (Duckett and Peel, 1978), this type of EDM has also been observed in *Haliptilon* (Vesk and Borowitzka, 1984). The *Bossiella* type has been more thoroughly documented and has been found to begin its nuclear association prior to meiosis. A smooth surface membrane system most likely comprised of ER surrounds stage 1 nuclei. EDM coats the
membranes and mitochondria associate with the nuclear envelope (Wilson, 1993). Within her survey, Wilson found that members of the Corallinaceae all of which possessed cell fusions, no secondary pit connections and uniporate conceptacles also possessed EDM. Those with marginal conceptacles (Bossiella and Calliarthron) exhibit Bossiella type EDM, while those with axial conceptacles (Jania, Corallina, and Haliptilon) exhibit Corallina type EDM. Melobesia, a non-geniculate coralline possessing cell fusions was expected to have EDM. Agee’s study (1995) however, showed that Melobesia lacks EDM throughout all stages of sporogenesis. However, members of the Melobesioideae (including the parasite Kvaleya epilaeve) also display multiporate conceptacles. This latter trait is present only in this subfamily and may suggest a more distant relationship to the other subfamilies with cell fusions. Hydrolithon, a member of the Mastophoroideae, also lacks EDM although it displays uniporate conceptacles and cell fusions. Choreonema also lacks EDM although its host Jania possess the Corallina type EDM. No other nuclear associations were observed in Choreonema other than perinuclear ER (PER) that will be discussed later.

**Golgi Apparatus: Structure/Function**

The Golgi apparatus (GA) in most red algae possess two unique characteristics: consistent association with mitochondria and the presence of appressed cisternae in the midregions during some stages of sporogenesis. In most eukaryotes, the GA is consistently associated only with ER at the cis face. Most genera of red algae, however, have in addition a mitochondria at the cis face (Pueschel, 1990). The GA-ER-mitochondria association is characteristic of corallines and has been observed in all stages of development in Choreonema. Close apposition of cisternae (Alley and Scott, 1977) have been repeatedly seen during sporogenesis in red algae studied in this laboratory and Choreonema is no exception. GA appear darkly stained during mucilage production in stage 2A and
cisternal compression occurs during fibrous vesicle production in late stage 2B.

The GA, during sporangial development, undergoes changes in number, morphology and vesicle type produced. The changes in general follow a similar sequence from genus to genus but not necessarily with the same timing. In Bossiella, (Wilson, 1993; Karnas, 1995), Melobesia (Agee, 1995), Haliptilon (Vesk and Borowitzka, 1984) and the non-corallines Levriniella (Kugrens and West, 1973a), Erythrocytis (Kugrens and West, 1974) Caulacanthus (Delivopoulos and Diannelidis, 1990), Gigartina (Tsekos, 1983b) and also Choreonema, GA bodies in early stages are initially rare, show a straight profile and appear inactive. As maturation progresses, the GA produces one to two other types of different vesicles. Bossiella, Melobesia and Haliptilon all exhibit a biphasic secretory pattern. The first vesicle produced contains an electron transparent material thought to be mucilage for use in the sporangial wall. Following this production, the GA take on a curved morphology and produce an electron dense vesicle commonly referred to as “osmiophilic vesicles”. The GA in Choreonema are triphasic in that they produce the first two vesicle types common to the other corallines but also produce a third type I call “fibrous vesicles”.

During production of the electron transparent vesicles thought to be mucilagous, the GA take on an electron dense appearance. This material does not remain in the cytoplasm of stage 2A sporangia, but is released to the outside of the cell. The uneven appearance of the cell wall and swollen aspects of the plasma membrane suggest active exocytosis to the developing mucilage layer and sporangial wall. Tsekos (1983b) found that what he called fibrillar vesicles in Gigartina teedii, thought to contain a mucilage-like substance, stained with alcian blue and alcian yellow, indicating they contain sulfated polysaccharides. These vesicles were exocytosed to the wall to form a mucilage layer. The presence of sulfated compounds in the mucilage layer may correspond to the high sulfur values found in the conceptacles of Choreonema during EDX analysis. A corresponding value was not found
in *Jania*.

The second vesicle type produced by the GA in *Choreonema* is the osmiophilic vesicle. These vesicles aggregate together, are strongly electron-dense, and may serve an adhesive function (Chamberlain and Evans, 1973; Pueschel, 1979; Vesk and Borowitzka, 1984). The appearance of these vesicles varies somewhat with the organism producing them and these differences are most likely caused by variation in chemical composition (Vesk and Borowitzka, 1984). The changed distribution of osmiophilic vesicles does not randomize until the GA begins production of its final vesicle type.

The third and final type of vesicle produced by the GA is unlike any described for other coralline sporangia. These vesicles, which I have termed fibrous vesicles, closely resemble (but on a smaller scale) the fibrous vacuoles produced by the GA in spermatia production in *Ptilota densa* (Scott and Dixon, 1973a). In *Ptilota*, they are used to discharge the spermatia from spermatangia and may also contribute to the mucilage layer and the adhesion of spermatia to the trichogyne. The vesicles found in *Choreonema* are unlike the fibrous vesicles described by Kugrens and West (1974) for *Erythrocytis saccata* as the ER does not contribute to their formation and they never achieve the size observed in *Erythrocytis*. In *Choreonema*, the fibrous vesicles become the most abundant vesicle type in the cytoplasm by stage 3, far outnumbering the osmiophilic vesicles. During fibrous vesicle production, the GA takes on a curved and swollen appearance, similar to observations of *Polysiphonia denudata* dictyosomes producing secretory vesicles (Alley and Scott, 1977). Peripheral cisternae enlarge while medial portions remain appressed. Unlike the secretory vesicles in *Polysiphonia*, fibrous vesicles remain independent of each other and do not combine to form large fibrous vacuoles.

**Endoplasmic Reticulum Associations**

All subfamilies examined within the Corallinaceae, with the exception of
Lithophylloideae and Melobesiodeae show perinuclear ER associations (PER) at some developmental stage of sporogenesis. In *Choreonema*, PER does not occur until late stage 2 and is not as tight an association as has been observed in other algae (Scott and Broadwater, 1990). At this stage, PER consists of 4-6 tracks of cisternae that circumscribe the nucleus and then produce spokes toward the periphery of the cell where it lies parallel to the plasmalemma. It is most obvious during late stage 2B when starch is produced along its cisternae.

Although no direct observations of production of the uniformly granular vesicles called grainy vesicles have been seen, similar vesicles produced in *Melobesia* (Agee, 1995) were produced late in stage 1 by the ER. These vesicles are often found near the ER and have never been observed associated with the GA. In *Choreonema*, grainy vesicles disappear shortly after late stage 2B; their function is unknown.

Unlike higher plants and green algae, starch is deposited in the cytoplasm of red algae (Pueschel, 1990). In the developing sporangia of *Choreonema, Melobesia* (Agee, 1995), *Haliptilon* (Vesk and Borowitzka, 1984), *Lithothrix* (Borowitzka, 1978) and *Amphiroa* (Dearstyne, 1994; Hanke, 1994) starch forms between the cisternae of ER. In most, starch formation occurs in stage 2 of sporogenesis; however, *Bossiella* has been shown to produce starch as early as late stage 1 (Wilson, 1993; Karnas, 1995). Instead of ER associations, however, in *Bossiella* starch is produced around large fibrous vacuoles. These vacuoles reduce in size until late stage 2 where they disappear and clusters of starch can be found between RER cisternae.

**Peripheral Tubules and Wall Formation**

Peripheral tubules appear in *Choreonema* only after full cleavage of the tetrasporangia. Thus, they are an indicator of stage 3 mature sporangia. Tubules have been observed in a number of species in the Ceramiales and in the Corallinales and are
perhaps found in all red algal sporangia (Azanzini and Honsell, 1984). These tubules have been shown to be continuous with the plasmalemma (Azanzini and Honsell, 1984; Karnas, 1995) and appear at the end of sporogenesis and may last until the onset of germination (Azanzini and Honsell, 1984). The peripheral tubule system appears fairly extensive in *Choreonema* and may have some association with the ER that runs along the edges of the plasmalemma.

It has been proposed that because these tubules increase the total surface area of the plasmalemma, they may serve in an osmoregulatory function prior to spore germination (Azanzini and Honsell, 1984). Peripheral tubules may also play a role in the development of spore wall which appears to be unique to coralline spores (Vesk and Borowitzka, 1984). The wall development was first described for corallines in *Haliptilon* (Vesk and Borowitzka, 1984) and has been observed in other corallines (Karnas, 1995; Agee, 1995), and appears similar in *Choreonema* also.

**Calcification**

Energy dispersive x-ray analysis proved the presence of calcium in the conceptacles of *Choreonema* but it is still unknown whether vegetative cells are calcified. Cabioch (1971b) indicated that these filaments are calcified, but this conclusion is not supported by Woelkerling (1987). However, neither researcher indicated a method for determining whether calcium was present or not. As expected, the calcite of *Choreonema* and *Jania* contain a small amount of magnesium. Members of the Corallinaceae deposit CaCO₃ in the form of calcite as massive deposits (Borowitzka et al., 1974; Borowitzka, 1981). Calcite (the rhomboidal form) commonly contains 7-30% magnesium carbonate (Lee, 1989). For example, the articulated coralline, *Bossiella*, has been reported to contain 12% magnesium carbonate (Pearse, 1972).

Calcification rates have been linked to photosynthesis in algae but this association
has been most actively studied in coral-zooxanthellae associations (Pearse and Muscatine, 1971; Goreau, 1959; Goreau and Goreau, 1959; 1960). One theory proposes that the uptake of carbon dioxide makes seawater more alkaline and enhances the precipitation of calcium salts. Seawater is generally maintained at pH 8.3. Raising pH slightly causes bicarbonate and free calcium to precipitate as calcium carbonate (Lee, 1989). Calcification rates are highest at the tips of both corals and geniculate red algae, where symbiotic zooxanthellae or algal cells with well developed plastids are rare (Pearse, 1972).

Translocation of photosynthetic products from basal regions to tip regions has been documented. Thus, organics produced by photosynthesis in the light may serve as the skeletal components or as general energy sources during calcification (Pearse, 1972). This supports the idea that *Choreonema* may be an active parasite as it is unable to photosynthesize (proplastids never mature); however, conceptacles show appreciable amounts of calcium. The energy required to utilize these minerals is likely to come from translocation of organics from *Jania*.

**Host Interactions**

Although labeled a parasite by some (Cabioch, 1980; Johansen, 1981) no real evidence of a parasitic interactions have been observed previously in studies on *Choreonema*. Woelkerling (1987) in a light microscopic study indicated that *Choreonema* was only endophytic on its host and that there were no indications of parasitism present. This is the first study to provide evidence that *Choreonema* is parasitic on its host *Jania*. The uniqueness of the interaction, however, leaves behind many questions as to the true nature of the parasitism. These include the degree of dependency and the evolutionary relationship between the host and parasite (ie: adelpho vs alloparsitism).

Endophytic and epiphytic algae range in dependence upon their hosts. Many are wholly independent and contain well developed chloroplasts. They use their host organism
as substrate for attachment but do not necessarily derive any nutrition from the host. *Polysiphonia lanosa* is an obligate epiphyte on *Ascophyllum nodosum*; however, it receives no nutritional support from its host (Harlin and Craigie, 1975). Other attached algal species including the corallines *Kvaleya epilaeva* on its host *Leptophytum laeve* (Adey and Sperapani, 1971), *Lesueuria minderiana* on its host *Metagoniolithon chara* (Woelkerling and Ducker, 1987), and *Ezo epiyessonense* on *Lithophyllum yessoense* (Adey et al., 1974) utilize haustoria to penetrate into host tissue and maintain only proplastids within their cells. Both possess either cell fusions (*Kvaleya* and *Lesueuria*) or secondary pit connections (*Ezo*) either of which may be used in a parasitic capacity (Goff, 1982). It is possible that cells of *Ezo* utilize the nuclear transfer observed in *Harveyella mirabilis* and other parasites to form multinucleate host cells and redirect genome production, however ultrastructural analysis has not been conducted on this species. The multinucleate state of *Choreonema* appears only in vegetative cells and nuclear transfer has not been observed between host and parasite.

Unlike *Kvaleya, Ezo* and *Lesueuria* whose haustoria penetrate only a few cells in their hosts, *Choreonema* utilizes many small cells to parasitize its host. Instead of using haustoria, vegetative cells within the host thallus cut off small cells I call conjoiner cells. Although superficially similar to the conjunctor cell seen in other red algal species, the conjoiner cell is not used for nuclear transfer. Instead, regions of these cells become very vacuolate and protrude toward host cells. When contact is made with the host, small tubules elongate and push through the host cell wall. Contact with the plasma membrane of the host occurs and it appears that the projections penetrate into host tissue although the fixation quality made it difficult to determine unequivocally the nature of the host-parasite interaction.

At initial contact the extending tubules are electron-transparent and no change in the host cell is obvious. In what we perceive as later stages, a reaction along the inner leaflet
of the host plasma membrane may be observed in which the membrane appears very vesiculate. Material along the inside of the host plasmalemma appears to slough off and ball up in the cytoplasm. During later stages, the projections from *Choreonema* contain an electron-dense material which extends into the host cell as an electron-dense layer inside the host plasma membrane or wall. At this stage, some host cells appear necrotic. Although the vegetative filaments produce a large number of conjoiner cells, unaffected host tissue appears fairly healthy although Cabioch (in Johansen, 1981) notes that the branch tips of *Jania* are often deformed.

The presence of only proplastids in spores and vegetative filaments strongly suggests that the conjoiner cells play a role in providing nutrition for *Choreonema*. This circumstantial evidence strongly suggests that *Choreonema* is an invasive active parasite. However, without labeling studies, it is impossible to known whether translocation of materials occurs from the host to *Choreonema*. Further studies utilizing radioisotope labeling need to be conducted before the parasitic status of *Choreonema* can be fully evaluated.

The original definition of adelphoparasites (organisms in the same tribe or family) (Goff, 1982) implies that *Choreonema* is an adelphoparasite on *Jania*. *Choreonema* also occurs on *Haliptilon* and *Cheilosporum*, both of which are included in the tribe Janieae (Johansen and Silva, 1978). In fact, possession of this parasite has been included as a defining character used to separate Janieae from the Corallinaceae. However, an observation of *Choreonema* growing on *Corallina officinalis* in the Mediterranean Sea (Johansen, 1981) exists and may undermine the use of this trait to define members of the tribe Janieae. Unlike all other adelphoparasites, *Choreonema* does not form secondary pit connections or cell fusions. Other adelphoparasites use these connections as a means of nuclear transfer and ultimately a way to take over control of the host's genome and its production of materials (Goff and Zuccarello, 1994). Although *Jania* also lacks
secondary pit connections, it does utilize cell fusions in making connections between non-kindred cells. Goff and Coleman (1985) have stated that secondary pit connections are an inherent character of all red algal parasites studied thus far, but this statement ignores the studies of corallines that utilize haustoria in their parasitic associations (Adey and Sperapani, 1971; Adey et al., 1974; Woelkerling and Ducker, 1987). *Choreonema's* use of specialized cells, however, is still a new and undocumented method of parasitism. The small cellular extensions could possibly be considered haustoria, however, they do not resemble the haustoria in size or shape that have been discussed in the above literature on parasitic corallines.

Another characteristic that separates *Choreonema* from other red algal parasites, is the development of reproductive and vegetative structures directly from the initial spore germination. Other red algal parasites utilize secondary pit connections to transform host cells into developing vegetative and or reproductive tissues for the parasite.

**Taxonomic Implications**

*Choreonema thuretii* originally was placed in the monophyletic subfamily Choreonematoideae based on the lack of both cell fusions and secondary pit connections, presence of tetrasporangial pore plugs and the presence of conceptacles that are one cell thick and lack epithallial cells (Woelkerling, 1987). Although this study has disproved the original description of conceptacle morphology, it has supported the other characters. In addition, it has provided several other unique characteristics that reinforce the establishment of its own subfamily. These include the unusual construction of the conceptacle, the possession of multinucleate cells; a condition never before observed in any coralline but common to parasitic algae (Goff, 1982) and the presence of specialized conjoiner cells that almost assuredly parasitize host tissues.

If the theory of adelphoparasitism holds up and *Choreonema* is closely related to its
geniculate hosts *Jania, Haliptilon, Cheilosporum,* and or *Corallina,* then one would expect it to share traits in common with these genera. However, many aspects of *Choreonema*’s biology suggest that it may be more distantly related. Because *Choreonema* is such a reduced form of coralline algae, it is impossible to determine whether it should be considered geniculate or non-geniculate form thereby excluding Johansen’s and Woelkerling’s primary character in defining subfamilies. However, both Cabioch/Chamberlain and Johansen/Woelkerling consider the type of cell connection to be phylogenetically important. *Choreonema* lacks both secondary pit connections and cell fusions even though all of its known hosts possess cell fusions. Cells of *Jania* are not multinucleate; vegetative cells of *Choreonema* are. Although cells of other parasite hosts may be multinucleate due to parasitic nuclear transfer, the multinucleate condition of *Choreonema* can not be considered to arise in the same manner since nuclear transfer does not occur and if it did, would make cells of *Jania* multinucleate. *Choreonema* has a conceptacle structure different than *Jania,* lacks EDM and even possesses a spore germination pattern (the bipolar *Naccaria* type) unlike that of *Jania* (which utilizes the discoid *Dumontia* type). This evidence suggests that *Choreonema* is more likely to be an alloparasite.

**Future Research**

Aspects of this study were limited by the relatively poor resolution of membranes and a new fixation is necessary in order to more clearly determine PER associations, proplastid development, and parasitic interactions via conjoiner cells. Furthermore, serial sections through a conceptacle may give us clearer understanding of the nature of the innermost cells and their role in the conceptacle construction. Finally, a new diagnostic description of the subfamily Choreonematoideae needs to written to take into consideration the findings of this study. Pulse-chase studies would be important to establish the
movement of organics across the conjoiner cell connections.

Also of interest to the question of parasitism in *Choreonema* would be research detailing electron microscopy studies of *Ezo* which possesses secondary pit connections to see if nuclear transfer occurs in this coralline parasite. More importantly, ultrastructural studies of *Kvaleya* and *Lesueuria* need to be conducted to determine if features similar to *Choreonema* exist for these coralline parasites that also lack secondary pit connections but possess cell fusions. In addition, DNA sequencing studies on *Choreonema* are needed to shed further light on the evolutionary relationships between this alga and its hosts as well as other coralline genera.
References


Table 1 Comparison of Taxonomic Schemes in the Corallinales

JOHANSEN (1981) and WOELKERLING (1988)

**Family Sporolithaceae**
- Heydrichia
- Sporolithon

**Family Corallinaceae**

<table>
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<th>Mastophoroideae</th>
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**Metagonioloithoideae**
- Metagoniolithon

**Lithophylloideae**
- Ezo
- Lithphyllum
- Tenarea
- Titanoderna

**Choreonematoideae**
- Choreonema

CABIOCH (1988) and CHAMBERLAIN (1978)

**Family Sporolithaceae**
- Heydrichia
- Sporolithon

**Family Corallinaceae**

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**Corallinoideae (con’t)**
- Geniculate Genera are in BOLD

55
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* indicates activities unique to tetrasporogenesis
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Diagram 1. Generalized life cycle of *Choreonema thuretii* including infection stages. This type of life cycle may be described as a trigenic (three generations), dimorphic (two morphologies), dibiontic (two collectable life forms, gametophyte and tetrasporophyte), diplohaplont (alternates between 1n and 2n stages) type life cycle.
Life cycle of *Choreonema thuretii*
Diagram 2. Schematic drawing compiling serial sections through a tetrasporangial conceptacle depicted in Fig. 8. Dotted lines indicate cells or connections not seen in Fig. 8. Colors indicate cell types. Blue = outermost (epithallial) cells, Green = innermost vegetative cells, Black = developing tetrasporangia, Light Blue = stalk cells, Red = intermediate cells, Blue (in the host thallus) = basal cells, Brown = host cells, Purple = vegetative filaments and conjoiner cells. Note the pit connections between innermost and outermost cells, between the intermediate cells and innermost cells, between intermediate and the basal cells and between the basal cells and the vegetative filaments. The vegetative filament at the base of the diagram is most likely derived from another conceptacle located on this host.
**Key to Abbreviations**

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<tr>
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<td>endoplasmic reticulum</td>
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<td>GA</td>
<td>Golgi apparatus</td>
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<td>starch</td>
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<td>TC</td>
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<td>tetraspore wall</td>
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<td>V</td>
<td>Vacuole</td>
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Figure 1. Aerial view of Santa Catalina Island. Specimens of *Choreonema thuretii* were collected on *Jania tenella* in September, 1994 and July, 1995 by Dr. Joseph Scott.

Figure 2. Stereoscope micrograph of *Choreonema* on *Jania*. Note the colorless conceptacles on the host thalli. x 30.
Figure 3. Scanning electron micrograph of spermatangial conceptacles of *Choreonema thuretii* on *Jania teriella*. All types of conceptacles (tetrasporangial, carposporangial and spermatangial) may be found on a single *Jania* thallus. x 180.

Figure 4. Scanning electron micrograph of a sporangial conceptacle. Note the exit pore and the similarities in the appearance of the outermost layer of cells of both *Jania* and *Choreonema*. x 460.

Figure 5. Scanning electron micrograph of a spermatangial conceptacle. Male conceptacles have a single exit pore and are easily differentiated from all other conceptacles by the appearance of a beak around the pore (arrow). x 690.
Figure 6. Transmission electron micrograph of *Choreonema* carposporangial conceptacle on a thallus of *Jania*. Necrotic cells in the center are the decaying carpogonium (arrow). Files of progressively maturing carposporangia are denoted by arrowheads. x 700.

Figure 7. Longitudinal medial section through a spermatangial conceptacle. Spermatangial initials (arrowheads) are formed from both the base and the roof of the conceptacle. Overall vegetative structure of male conceptacles is the same as described for carposporangial and tetrasporangial conceptacles. x 1,040.

Figure 8. Longitudinal section through a tetrasporangial conceptacle illustrating basal cells (B) and part of an elongate vegetative filament (*). Note pit connection (arrow) from the exterior layer of the conceptacle to a cell which is pit connected to a basal cell in later sections. Only stage 1 and stage 2 tetrasporangia are visible. This conceptacle is the same one from which Diagram 2 was constructed. x 1,110.
Figure 9. Longitudinal section through a tetrasporangial conceptacle showing stalk cells (S) that produce the developing tetrasporangia. Below the stalk cells lie intermediate cells (I) that ultimately connect to the basal cell (B). x 1,616.

Figure 10. Multi-nucleate basal cell (B) shown in Diagram 2 and Fig. 8. Note the concentric cisternae of endoplasmic reticulum (arrowheads). x 8,280.

Figure 11. Basal cell nucleus (N) and organelles. These cells are replete with endoplasmic reticulum (ER), mitochondria (M) and vacuoles (V) containing a reticulated material. x 12,960.
Figure 12. Longitudinal section through a tetrasporangial conceptacle. Each of the three developing tetrasporangia is a single cell. Note the presence of a pit connection (PC) from a vegetative filament to an outermost epithallial cell. A, B and C illustrate areas where the overlying vegetative cell appears to end. Closeups of these areas in Figs 13-15 show that this vegetative cell is continuous. x 2,525

Figure 13. Closeup of A showing the continuation of this cell along the left side of the conceptacle. x 13,680.

Figure 14. Closeup of B showing the continuation of this cell along the top of the conceptacle. x 6,160.

Figure 15. Closeup of C showing the continuation of this cell along the right side of the conceptacle. x 13,680.
Figure 16. Cross-section through the top of a tetrasporangial conceptacle. Note the pit connections between the innermost and outermost (epithallial) layer of cells (arrowheads). It is extremely difficult to access the nature of the innermost cells due to their irregular shape, lack of distinct walls and vacuolate makeup. These cells also show close association with the developing sporangia (*). x 1,647.
Figure 17. Glancing longitudinal section through a tetrasporangial conceptacle. Innermost vegetative cells (*) are extremely large and vacuolate and form the underlying support structure for the conceptacle. Note also the connections between the vegetative cells and the intermediate cells (I) which ultimately connect to the basal cells (B) and the appearance of partial pit connection (arrow). x 1,340.

Figure 18. Vegetative filaments along the outside edges of developing sporangia may also derive other filaments as evidenced by the presence of primary pit connection (arrow). x 5,780.

Figure 19. Innermost vegetative cells contain many mitochondria (M), vacuoles (V) and extensive endoplasmic reticulum (ER) and may possess one or more nuclei (N). x 12,240.

Figure 20. Vegetative cells derive the outermost layer of cells. These cells have a thickened cell wall and reduced cytoplasm (arrowheads). Pit connections (PC) between vegetative cells and the outermost layer are commonly seen. x 5,780.
Figure 21. Longitudinal section through multiple tetrasporangial conceptacles stained with toluidine blue and viewed under Nomarski DIC optics. Note the long vegetative filaments of *Choreonema* within the host (*). x 650.

Figure 22. Vegetative filaments of *Choreonema* (delimited by arrows) can be extremely long (up to 115 µm in length) and narrow. x 1,160.

Figure 23. Vegetative filaments of *Choreonema* may be multi-nucleate (N) with proplastids (p), numerous mitochondria (M) and vacuoles (V). x 1,465.

Figure 24. Vegetative filaments of *Jania* are easily distinguished from *Choreonema* by the presence of fully developed chloroplasts (*), numerous starch grains (not shown but seen in Fig. 21) and a large central vacuole (V). x 11,500.
Figure 25. Longitudinal section through a tetrasporangial conceptacle showing a stage 3 tetrasporangium mucilaginous extensions that constitute the pore plugs (pg) that collectively block the pore (not shown). Possession of uniporate tetrasporangial conceptacles blocked by pore plugs is one of the defining traits of the Choreonematoideae. x 1,160.

Figure 26. Scanning electron micrograph of a tetrasporangial conceptacle showing the exit pore (arrow). x 3,000.

Figure 27. Closeup of the conical layered extensions of mucilage that comprise the pore plug (pg). x 2,040.
Figure 28. Longitudinal section through a tetrasporangial conceptacle. The three stage 2 tetrasporangia are pit connected to stalk cells (S) at their base. Stalk cells are pit connected to intermediate cells (I) that may be multinucleate (*). x 1,070.

Figure 29. Stalk cells (S) have dense cytoplasm, lack a cell wall and generally are more regular in shape than intermediate cells (I). x 6,800.

Figure 30. Stalk cells (S) contain few organelles but display a slightly less electron dense area around the pit plug (arrowhead). x 14,400.
Figure 31. Stage 1 tetrasporangia (TC) which are usually located near the center of the conceptacle, have elongate, irregular shapes and lack cell walls. The cytoplasm of these cells are dense but organelles are relatively scarce. x 3,030.

Figure 32. Stage 1 tetrasporangia become more vacuolate as they mature. Vacuoles (V) appear to be exocytosed after meiosis. x 10,200.

Figure 33. Stage 1 nuclei (N) at this stage are characterized by distinct nuclear pores and the nucleoli (n) that are appressed against one side of the nucleus (see Fig. 31). Rough ER (ER) and mitochondria (M) are present in the cytoplasm. x 10,120.
Figure 34. Cytoplasm of stage 1 tetrasporangium with lipid droplet (L). Mitochondria (M) are associated with the Golgi apparatus (GA). x 62,890.

Figure 35. Early tetrasporangial wall (TW) formation in a stage 1 tetrasporangium. Note the presence of peripheral endoplasmic reticulum (ER). x 100,700.

Figure 36. Section through a meiotic nucleus (N). Nuclear pores become extremely distinct. At this stage grainy vesicles (g) are present and the cell is developing the scalloped appearance (arrowheads) characteristic of early stage 2A tetrasporangia. x 23,560.

Figure 37. Prophase I nucleus showing synaptonemal complex (*). x 21,080.
Figure 38. Early stage 2A tetrasporangium with cleavage furrows (*). Appearance of a completed cleavage furrow is due to plane of section. GA (arrows) are producing mucilage to be exocytosed to the cell wall. ER (arrowheads) is present in the cytoplasm near the (N). x 7,820.

Figure 39. Closeup of Golgi apparatus (GA) (with mitochondrial association (M)) producing mucilage. Extensive endoplasmic reticulum (ER) in the nuclear region is also apparent. x 38,080.
Figure 40. Substage 2B tetrasporangium showing that cleavage furrows (arrowheads) extend more than one third of the way into the cells from any direction. Also note the clusters of osmiophilic vesicles (*). These cells lack the vacuoles and mucilage seen in Fig. 38. x 4,040.

Figure 41. Stage 2B nucleus (N) with parallel tracts of rough endoplasmic reticulum (ER) near but not encircling it. Nuclear pores (arrows) are present but less well defined than in Figs 36, 37. x 29,760.

Figure 42. Units of the Golgi apparatus (GA) in stage 2B show a curved profile and produce osmiophilic vesicles (o). x 62,890.

Figure 43. Stage 2B tetrasporangium with cleavage furrows. Large numbers of osmiophilic vesicles (o) are present in clusters throughout the cell. Note the thick mucilage layer (*) and the tetrasporangial wall (arrows). x 23,560.
Figure 44. Mid stage 2B tetrasporangium. Note the presence of fibrous vesicles (*) produced by the GA and starch (arrowheads) produced along the tracts of ER. x 3,050.

Figure 45. Mid Stage 2B with Golgi (GA) producing fibrous vesicles (f). Starch (s) is produced between cisternae of parallel endoplasmic reticulum (ER) tracks near the nucleus (N). x 49,280.

Figure 46. Typically ER cisternae with starch (s) appear to surround the nucleus (N) (perinuclear ER association) then extend out as spokes toward the cell periphery (arrows). x 7,480.

Figure 47. Mid stage 2B tetrasporangium with Golgi apparatus (GA) exhibit appressed mid cisternae and swollen peripheral cisternae during production of fibrous vesicles (f). Note the ER tracks (ER) with associated starch (s) that comprise the spokes described in Fig. 46. x 49,280.
Figure 48. Late stage 2C tetrasporangium characterized by randomization of all vesicles and starch in the cytoplasm. x 5,050.

Figure 49. Late stage 2C tetrasporangium exhibiting disassociation of starch (*) from ER (arrows). The PER association is still present (arrowheads). x 23,560.

Figure 50. Late stage 2C tetrasporangium showing arrested cleavage. Proplastids (p), which are rarely detectable, are present along with ER (arrowheads) near the nucleus. x 8,740.
Figure 51.  Fully cleaved stage 3 tetrasporangium with three tetraspores observable. Cleavage furrows (*) are narrowed and irregular in outline. Three cleavage furrows are present, however, this plane of section only allows visualization of two. All vesicles are randomized in the cytoplasm. x 6,090.

Figure 52.  Cell periphery of a tetraspore partially bounded by a cleavage furrow (double arrowhead). Peripheral tubules (arrows) extend perpendicular from the plasmalemma into the cytoplasm. Just outside of the plasma membrane, a developing tetraspore wall (arrowheads) is apparent. Note also the mucilage layer (m), the tetrasporangial wall (TW), osmiophilic vesicles (o), fibrous vesicles (f), and starch (s). x 42,560.

Figure 53.  Stage 3 tetraspore with peripheral tubules (arrows) ranging in length from 0.03 - 0.32 \( \mu \text{m} \). Note the apparent continuity of the lumen of the tubule with the cell exterior (arrowhead). Note the tetraspore wall (Ts), and the tetrasporangial wall (TW). x 79,440.
Figure 54. Whole carposporangial conceptacle with basal cells (arrowheads) and a central fusion cell (arrow) formed from the merging of auxiliary cells and intermediate cells. Gonimoblast filaments composed of stages of carposporangia in succeeding developmental stages can be observed from the base of the conceptacle (less mature) to the apex (mature). Note at the base of the conceptacle the presence of *Jania* cells with starch (*) and a large central vacuole (V). $x$ 2,013.
Figure 55. Fusion cell (F) in carposporangium. These cells are large and multinucleate due to the convergence of many intermediate and auxiliary cells. x 3,030.

Figure 56. Higher magnification of a fusion cell showing six nuclei, large reticulated vesicles (*) and fibrillar vacuoles (arrow) present. x 6,460.

Figure 57. Portion of a fusion cell showing a pit connection (PC) to a gonimoblast cell (arrow). x 13,680.
Figure 58. Longitudinal section through a carposporangial conceptacle showing succeedingly maturing carposporangia. A small section of the fusion cell is still present near the base of the conceptacle. Two gonimoblast cells (*) have just divided and are in the process of cytokinesis to produce stage 1 carposporangia as denoted by the appearance of cleavage furrows (arrows). x 1,780.

Figure 59. Closeup of dividing gonimoblast cell from Fig. 58. Note the arrangement of vesicles across the center of the cell and the small cleavage furrows (arrowhead). x 34,720.

Figure 60. Closeup of dividing gonimoblast cell from Fig. 58. To be noted here are the membranous vesicles (arrowheads) in the cytoplasm. x 32,000.
Figure 61. Stage 1 carposporangium. Note the large number of vacuoles, the presence of lipid droplets (L), numerous mitochondria (arrows) and lack of a carposporangial wall. x 10,070.

Figure 62. Early stage 2A carposporangium. GA are producing mucilage (arrows) for exocytosis to the cell wall resulting in a frilled appearance to the cell periphery. Grainy vesicles (g) are present. x 15,840.

Figure 63. Early stage 2B carposporangium characterized by the Golgi apparatus (GA) are producing osmiophilic vesicles (*) in clumped distributions. ER (arrow) is present near the nucleus (N). x 6,460.

Figure 64. Mid stage 2B carposporangium. GA are producing fibrous vesicles (*) concurrent with production of starch along the spoked ER channels (arrowheads) radiating from the nucleus (N). x 7,480.
Figure 65. Stage 2A carposporangium showing the Golgi apparatus (GA) producing mucilage and exocytosis of this material to the mucilage layer and the carposporangial cell wall (CW). Grainy vesicles (g) are also present. x 62,890.

Figure 66. Early stage 2B carposporangium with the Golgi apparatus (GA) producing osmiophilic vesicles (o). x 62,890.

Figure 67. Late stage 2B carposporangium with the Golgi apparatus (GA) producing fibrous vesicles (f). Golgi-mitochondrial associations are still present. Note the fibrillar nature of the mucilage sheath (arrow) x 42,560.

Figure 68. Late stage 2 carposporangium showing starch (s) production along rough endoplasmic reticulum (ER) tracts. Fibrous vesicles (f) and osmiophilic vesicles (o) are also present. x 62,890.
Figure 69. Stage 3 carpospore. All vesicles including starch are randomized in the cytoplasm. x 6,460.

Figure 70. Stage 3 carpospore showing a proplastid (p), nucleus (N), starch (s) and a random distribution of vesicles. x 42,560.

Figure 71. Closeup of stage 3 carpospore showing peripheral tubules (arrows) extending perpendicular from the plasmalemma into the cytoplasm. Peripheral ER can also be seen (*). A developing carpospore wall (arrowheads) is also present. Note also the appearance of osmiophilic vesicles (o), fibrous vesicles (f), starch (s) and lipid droplets (L) in the cytoplasm. x 42,560.

Figure 72. Closeup of stage 3 carpospore with peripheral tubules (arrow) and the developing carpospore wall (arrowheads), mucilage layer (*) and carposporangial wall (CW). x 99,300.
Figure 73. Vegetative filament at the base of a conceptacle illustrating the production of conjoiner cells (*) and their position alongside or near host cells (H). x 2,626.

Figure 74. Closeup of the Fig. 73 showing the pit connection (PC) between the vegetative filament and a conjoiner cell (C) and its parasitic interaction (arrows) on a host cell (H). x 11,960.
Vegetative filaments of *Choreonema* are very long (17.0 μm) and produce a number of small cells (1.8 μm) we call conjoiner cells (*) within the host thallus. x 6,460.

Conjoiner cells connected to two *Jania* cells (H1 and H2). Conjoiner cells have very few organelles and are readily differentiated from the host cells. Attachment points are characterized by haustoria-like projections that seem to extend across the cell wall of the host. x 16,560.

Conjoiner cell (C) with multiple connections (arrows) with a single host cell (H). x 13,680.

Two or more conjoiner cells (C1 and C2) may attach to a single host cell (H). Note the multiple finger-like projections from each protuberance (arrows). x 9,200.
Figure 79. Conjoiner cell (C) with two connections with the *Jania* host (H). The protuberances producing the filamentous projections are very vesiculate (*). Vesicles may be electron-translucent or fibrillar. x 6,640.

Figure 80. Recently established conjoiner (C) extensions as deduced from their moderate electron opacity and the healthy condition of the host cell (H). x 23,560.

Figure 81. Conjoiner (C) extensions showing apparent penetration of host membrane (arrows). The meandering of the extension and poor membrane preservation make it difficult to ascertain the extent of the invasion within the host (H) cytoplasm. x 42,560.

Figure 82. A conjoiner cell (C) with extensions (arrows) apparently penetrating the membrane near starch vesicles of the host (H). x 42,560.
Figure 83. Conjoiner connection considered to be a later stage of infection inferred from the vesiculate plasma membrane (arrows) of the host (H). The membrane disturbance appears to be a response by the host to the presence of the conjoiner cell (C). x 23,560.

Figure 84. Late stage connection a deduced by the presence of an osmiophilic substance (arrow) in the extensions as well as in the host cell (H). Host cells at this stage appear necrotic. x 13,680.

Figure 85. Closeup of an extension with osmiophilic material (arrow) and showing the continuation of similar material (arrowheads) in the host (H). Also note the possible origin (*) of this material in the conjoiner cell (C) and in Fig. 84. x 42,560.

Figure 86. A membranous mass along the inside of a plasma membrane of a necrotic host cell (H). The mass appears to be an accumulation of vesiculate membranes such as those in Fig. 83. x 106,000.
Figures 87-89. Electron dispersive x-ray analysis spectrograph of data from *Jania tenella*. The x-axis equals 0.0-10.2 KeV (a range normally used for biological samples) and the y-axis is an arbitrary scale set for the greatest resolution of the elements found. The first peak seen for calcium corresponds to a vacancy filled in the $K_{\alpha}$ orbital surrounding the element while the second lower peak for calcium corresponds to a vacancy filled in the $K_{\beta}$ orbital. To be noted in these graphs are the high calcium peaks and the relatively low amounts of sulfur. Peaks for copper are caused by the holding stub.
Figures 90-93. Energy dispersive X-ray analysis spectrograph of *Choreonema thuretii*. The x-axis equals 0.0-10.2 KeV (a range normally used for biological samples) and the y-axis is an arbitrary scale set for the greatest resolution of the elements found. The first peak seen for calcium corresponds to a vacancy filled in the $K_{\alpha}$ orbital surrounding the element while the second lower peak for calcium corresponds to a vacancy filled in the $K_{\beta}$ orbital. Note the moderately high calcium peak and the high readings for sulfur not seen in the graphs of *Jania*. The sulfur readings may be caused by sulfated polysaccharides present in the thick mucilage layers of sporangial conceptacles. Readings for copper, and in “nodule 1” for aluminum, chloride and iron are due to the stub and the double sided tape.
VITA

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Born on December 8, 1969 in Elmira, NY, grew up in rural New Hampshire graduating Hollis Area High School in 1988. Entered the University of New Hampshire and majored in Biology with a concentration in Marine and Freshwater Science. During the junior year (1990-91), entered Northeastern University’s East-West Marine Biology Program and studied in Friday Harbor Labs, WA, Discovery Bay Marine Lab, Jamaica and at the Marine Science Center, Nahant, MA. After graduating UNH in December of 1991, went on to employment at Harvard University’s Department of Microbial Ecology as a research technician. While at Harvard University, collaborated on three papers with research faculty. During October of 1992 while still at Harvard University, began work as a microbial consultant for Massachusetts Institute of Technology’s Microsystems Group on their large scale deionized water facility and developed a water quality monitoring program. Married Gregg J. LaRoche in November of 1993 and entered the College of William and Mary’s Master of Arts program in biology in January of 1994. Received teaching assistantship for the second year of study. Commencing December, 1995. Relocating to Acton, MA in Fall, 1995.