

GELIDIALES (RHODOPHYTA, RED ALGAE) IN BRITISH COLUMBIA AND
NORTHERN WASHINGTON: TAXONOMY, MORPHOLOGY, DEVELOPMENT
AND LIFE HISTORY

by

DAWN ELIZABETH RENFREW

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Department of Botany
The University of British Columbia
Vancouver, Canada

Date Aug. 1988

ABSTRACT

A study of the red algal order Gelidiales in British Columbia and northern Washington was conducted. Gelidiales are represented in the study area by four species, *Gelidium coulteri*, *G. purpurascens*, *G. vagum* and *Pterocladia caloglossoides*, and a key to identify the taxa is provided. Earlier reports of *G. crinale*, *G. pusillum*, *G. robustum* and *G. sinicola* from British Columbia and northern Washington have been shown to be misidentifications of the other taxa and are excluded from the flora. The occurrence of *G. vagum* in the British Columbian flora is significant because it appears to have been introduced from the northwest Pacific and now has a disjunct distribution, occurring in Japan and China and on two islands in the Strait of Georgia, British Columbia. *Gelidium vagum* and the Gulf of California species, *G. johnstonii*, are considered to be sister species. They share the unique character of monoecy (i.e. unique in the genus) and a morphological resemblance, but differ in several other characters, including arrangement of cortical cells, shape and flattening of tetrasporangial stichidia, shape of stichidial apices and position of the apical initial with respect to the adjacent cortex, presence/absence of an apical furrow and sterile margin in tetrasporangial stichidia and presence/absence of intact third order filaments in mature cystocarps.

The life history of *Gelidium vagum* was completed in culture, only the second *Gelidium* species for which this has been accomplished. *Gelidium vagum* has a triphasic *Polysiphonia*-type life history with isomorphic (monoecious) gametophytes and tetrasporophytes, and carposporophytes growing on gametophytes. The rare

occurrence (*G. purpurascens*, *G. coulteri*) or absence (*Pterocladia caloglossoides*) of gametophytes in the field and the lack of successful completion of a life history in culture suggests that, whereas these species may occasionally complete a *Polysiphonia*-type life history *in situ*, vegetative or apomictic mechanisms are more important in maintaining field populations. Chromosome counts of $n=14-15$ were made on undivided tetrasporangia of *G. vagum*.

The pattern of spore germination in *Gelidium coulteri*, *G. purpurascens* and *G. vagum* is similar for carpospores and tetraspores. A single germ tube grows from the spore, the entire cytoplasmic contents of the spore evacuate into the germ tube and a wall cuts the germ tube off from the empty spore. The germ tube divides unequally to form a concave and a fusiform cell. The primary attachment rhizoid forms from a derivative of the concave cell and the sporeling apical cell forms from a fusion cell derivative. As apical organization is established, the subapical cell produces two lateral periaxial cells.

Carpogonium and carposporophyte development was followed in *Gelidium purpurascens* and *G. vagum*. The functional carpegonium is intercalary. Non-functional carpegonia divide transversely with a concave wall and become sessile and degenerate. The fertilized carpegonium consistently forms a fusion cell by fusing with adjacent cortical cells. Fusion cell lobes cut off gonimoblast filaments that send out processes that fuse with haploid gametophyte cells of nutritive chains. Carposporangia are produced terminally and laterally by gonimoblast cells and protrude into two locules that have formed as the cortex is pushed away from the plate of second order filaments. Carpospores are released

through a single ostiole in each locule.

Spermatogenesis, tetrasporogenesis and rhizine morphology were studied ultrastructurally for the first time in Gelidiales. In spermatangial development, apical chloroplasts in the spermatangial mother cell are cut off by a furrow, a mechanism previously not reported in red algae for exclusion of chloroplasts from spermatangia. As the spermatangium matures, spermatangial vacuoles form from the coalescence of vesiculated endoplasmic reticulum and dictyosome-derived vesicles. Spermatangial vacuoles are discharged from the spermatangium just prior to the release of the spermatium.

Stages of tetrasporangium development are marked by wall development. Prior to karyokinesis, the young tetrasporangium is dominated by a large nucleus. Following karyokinesis, chloroplasts, starch grains and dictyosomes increase in abundance, a wall layer is deposited, and dictyosomes undergo a series of morphological changes from small and flat, to large and flat producing fibrillar vesicles, to hemispherical and producing cored vesicles. At cytokinesis an electron dense wall layer is deposited around the tetrasporangium and in the cleavage furrow, and cored vesicles secrete a mucilage-like material inside this wall layer.

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CHAPTER I. INTRODUCTION

A. TAXONOMIC HISTORY

Gelidiales Kylin (1923: 132) currently encompass the single family Gelidiaceae Kützinger (1843: 390) which contains 11 genera: *Acanthopeltis* Okamura (in Yatabe, 1892: 157), *Becherella* Kylin (1956: 139), *Gelidiella* Feldmann *et* Hamel (1934: 529), *Gelidium* Lamouroux (1813: 128), *Onikusa* Akatsuka (1986a: 63), *Porphyroglossum* Kützinger (1847a: 775), *Pterocladia* J. G. Agardh (1852: 482), *Pterocladiastrum* Akatsuka (1986c: 55), *Ptilophora* Kützinger (1847b: 25), *Suhria* J. G. Agardh *ex* Endlicher (1843: 41) and *Yatabella* Okamura (1900: 1). Historically, a heterogeneous assemblage of genera (now included in four orders) was ascribed to the family (Kützinger, 1849; Schmitz, 1889). A historical summary of genera included in Gelidiaceae in seven major classifications is presented in Table 1.

The family Gelidiaceae was erected by Kützinger (1843: 390, 405 as "Gelidieae"; vide Silva 1980: 83) to encompass cartilaginous, pinnately branched plants, with fibrous internal structure, exserted cystocarps, small globose spermatia and cruciate tetrasporangia on distinct tetrasporangial branches. The family originally comprised *Acrocarpus* Kützinger (1843: 405), *Ctenodus* Kützinger (1843: 407), *Echinocaulon* Kützinger (1843: 405) and *Gelidium*, with the later addition (Kützinger, 1849) of *Polycladia* Montagne (1847: 378), *Thysanocladia* (Endlicher) Lindley (1846: 25), *Delisea* Lamouroux (1819: 41), *Chondrodon* Kützinger (1847b: 5) and *Phacelocarpus* Endlicher *et* Diesing (1845: 289), and the substitution of *Euctenodus* Kützinger (1847b: 5) for *Ctenodus*. J. G. Agardh's (1851) concept of Gelidiaceae

(as Gelidieae) was narrower, including only the genera *Gelidium* [composed of the subgroups *Acrocarpus* (= *Gelidium* in part), *Echinocaulon* (= *Gelidiella*) and *Gelidium*], *Pterocladia* (newly erected), *Suhria* and *Ptilophora*, all genera currently belonging to the family. *Wurdemannia* Harvey (1853: 245) was added (with a query) because of a similarity in thallus construction to *Gelidium* (J. G. Agardh, 1876). Schmitz (1889) devised a classification scheme based on thallus construction and cystocarp construction and development and recognized in Gelidiaceae 14 genera in five tribes. Schmitz's family concept was broad and heterogeneous, and all genera in four of the tribes, i.e. Binderelleae [including *Binderella* Schmitz (in Engler and Prantl, 1897: 342) and *Choreocolax* Reinsch (1874-1875: 61)], Harveyelleae [including only *Harveyella* Schmitz et Reinke (1889: 28)], Wrangelieae [including *Wrangelia* C. A. Agardh (1828: 136), *Atractophora* H. M. Crouan et P. L. Crouan (1848: 371) and *Naccaria* Endlicher (1836: 6)] and Caulacanthaeae [including only *Caulacanthus* Kützinger (1843: 395)] were transferred eventually to other families. Schmitz did recognize, however, the similarities of gelidiaceous plants, and he placed *Gelidium*, *Pterocladia*, *Suhria*, *Porphyroglossum*, *Ptilophora*, *Acropeltis* Montagne (1837: 355) and *Schottmullera* Grunow (in Schmitz 1889: 440; *nomen nudum*) in the tribe Gelidieae and excluded *Wurdemannia* from Gelidiaceae. *Schottmullera* was renamed *Acanthopeltis* by Okamura (in Yatabe, 1892) and, along with *Spencerella* Darbishire (1896: 199), it was added to Gelidiaceae (Schmitz and Hauptfleish, 1897).

Oltmanns (1904) removed *Harveyella*, *Wrangelia*, *Atractophora* and *Naccaria* to Wrangeliaceae (Gigartinales). De Toni (1924) apparently disagreed with Oltmanns' removal of Wrangeliaceae, because he added *Haliacantha* J. G. Agardh (1899:

109) and *Gulsonia* Harvey (1855: 334) to the tribe Wrangelieae in the Gelidiaceae. He also added *Yatabella* and *Spencerella* to the tribe Gelidieae. *Choreocolax* was removed to Cryptonemiales (Sturch, 1926), *Harveyella* and *Caulacanthus* were transferred to Gigartinales (Sturch, 1926 and Feldmann and Hamel, 1934, respectively) and the new genus *Gelidiocolax* Gardner (1927b: 340) was added to Gelidiaceae. Feldmann and Hamel (1934) renamed *Echinocaulon* *Gelidiella*, because the name *Echinocaulon* was a later homonym for a genus of Polygonaceae. Kylin (1956) revised the contents of Gelidiaceae as it had developed from Schmitz, transferring *Binderella* to Gigartinales, *Haliacantha*, *Gulsonia* and *Spencerella* to Ceramiales, and adding *Beckerella*. Fan and Papenfuss (1959) removed *Gelidiocolax* to Cryptonemiales. Fan (1961) placed *Gelidiella* in its own family, Gelidiellaceae, based on the absence of rhizines and apparent lack of a sexual generation.

Recently, Santelices and Montalva (1983) merged the monotypic genus, *Acropeltis*, characterized by shield-shaped tetrasporangial stichidia, with *Gelidium*, stating that this character "has no taxonomic significance in presently accepted schemes of classification of the Gelidiaceae." As a result of studies of vegetative characters that emphasized surface cell morphology, Akatsuka (1986a) erected *Onikusa*, based on *Gelidium pristoides* (Turner) Kützinger (1849: 786) from South Africa. Also using morphological characters with an emphasis on cortical cell morphology Akatsuka (1986c) erected *Pterocladiastrum*, based on the "Robust" and "Poor Knights" forms of *Pterocladia lucida* from New Zealand.

The position of Gelidiaceae has fluctuated between inclusion in Nemaliales Schmitz

in Engler (1892) and recognition as an independent order, Gelidiales Kylin (1923). When Gelidiaceae was erected, Kützting (1843) included it in the order Periblasteae. Schmitz (1883) placed Gelidiaceae in its own order, but later (Schmitz, 1889) included it in Nemaliales (as "Nemalioninae"), where it remained until Kylin (1923) raised it to ordinal rank, coordinate with Nemaliales (as "Nemalionales"; see Nicolson and Norris, 1983 for correct spelling and use of Nemaliales), Cryptonemiales Schmitz in Engler (1892), Gigartinales Schmitz in Engler (1892), Rhodymeniales Schmitz in Engler (1892) and Ceramiales Oltmanns (1904). Kylin saw Nemaliales and Gelidiales as similar in that the fertilized carpogonium in both was the starting point for the gonimoblast. According to Kylin (1923), auxiliary cells were absent in Nemaliales but present in Gelidiales, functioning in a nutritive capacity. Kylin noted that Gelidiales were believed to be diplobiontic, in contrast to the supposed haplobiontic Nemaliales, although he specifically refrained from using life history characters to separate the orders.

However, misinterpreting that Kylin (1923) had elevated Gelidiaceae to ordinal rank on the basis of life history differences (diplobiontic in Gelidiales versus haplobiontic in Nemaliales), Dixon (1961) returned Gelidiaceae to the Nemaliales (as "Nemalionales"). He cited the questionable existence of a regular *Polysiphonia*-type life history (Dixon, 1961) in some *Gelidium* and *Pterocladia* species, based on the rare occurrence or absence of generations, usually gametophytes, and the lack of reports of sexual plants for *Gelidiella* species, as an argument against regarding Gelidiaceae as strictly diplobiontic. Dixon also pointed out that some members of Nemaliales (as Nemalionales) (e.g. *Bonnemaisonia*) appeared to be diplobiontic. Hence life history type no longer

could be used to separate Gelidiales from Nemaliales. Papenfuss (1966) argued, however, that ordinal status for Gelidiales was warranted as they were characterized uniquely by nutritive chains, the production of only two pericentral cells and *Gelidium*-type spore germination.

Gelidiales contained the single family Gelidiaceae until Fan (1961) erected a second family, Gelidiellaceae, to contain species of *Gelidiella* (discussed above). The report of rhizines in *Gelidiella calcicola* (Maggs and Guiry, 1987) and cystocarps in a *Gelidiella* species from India (Sreenivasa Rao and Trivedi, 1986, cited in Maggs and Guiry, 1987) contradict Fan's (1961) characters for separation of the family and Maggs and Guiry (1987) merged Gelidiellaceae with Gelidiaceae.

B. CHARACTERIZATION OF GELIDIALES

At present, Gelidiales is recognized as an independent order (Santelices, 1974; Garbary *et al.*, 1982; Gabrielson and Garbary, 1986, 1987; Hommersand and Fredericq, 1988). The order appears monophyletic (Gabrielson and Garbary, 1986, 1987), having several unique characters that set it apart from other orders. Gelidiales are uniaxial, pseudoparenchymatous, have pit plugs with a single plug cap (Pueschel and Cole, 1982), and show "*Gelidium*-type" spore germination (Chemin, 1937) with germling development proceeding directly to prostrate or upright axes without an intervening discoid stage (Boillot, 1963). Axial cells produce two periaxial cells at 180° (Fan, 1961), and inner cortical cells produce elongate, thick-walled, unicellular rhizines that grow basipetally. *Gelidium*-type spore germination and sporeling development (Papenfuss, 1966) and rhizines are

features unique to Gelidiales (Fan, 1961; Santelices, 1974).

Rhizines, also referred to as rhizoids and hyphae (Feldmann and Hamel, 1936; Dixon, 1958; Fan, 1961), are unique anatomical features of the vegetative thallus of Gelidiales. Present in all genera of Gelidiales, rhizines are found throughout the thallus in all except *Gelidiella* where they are reported to occur only near attachment pads (Maggs and Guiry, 1987). Rhizines differ from multicellular rhizoid filaments that grow between medullary cells of some other genera of red algae, in that gelidioid rhizines are unicellular. This distinguishes rhizines *sensu* Feldmann and Hamel, 1936) from external attachment rhizoids.

Spermatangia are produced by the transverse division of spermatangial mother cells (Tazawa, 1975), a character unique to Gelidiales at the ordinal rank (Gabrielson and Garbary, 1987). In a recent study of pre- and post-fertilization events in *Gelidium peridifolium* Hommersand and Fredericq (1988) revised some previous interpretations. According to Hommersand and Fredericq the carpogonium is intercalary and has associated chains of nutritive cells. An auxiliary cell *sensu* Drew (1954) is absent, because as the fertilized carpogonium enlarges, it makes non-obligatory fusions with adjacent chains of nutritive cells to form a fusion cell. The nutritive cells fused with are not "specified" (*sensu* Drew), and thus cannot be interpreted as auxiliary cells. The fusion cell initiates a filamentous carposporangium-producing gonimoblast within a pericarp. Tetrasporangia are cruciate, irregular cruciate or tetrahedral (Guiry, 1978). The life history is presumed to be of the *Polysiphonia*-type (Santelices, 1974), but has been completed for only two species of *Gelidium* (Macler and West, 1987; van der

Meer, pers. comm.). The life history of the type species of *Gelidium* still needs to be followed in culture.

C. DIFFERENTIATING GELIDIUM AND PTEROCLADIA

The most speciose and geographically widespread genera of Gelidiaceae, *Gelidium* and *Pterocladia* (Santelices, 1974), are both represented in the British Columbia flora. *Gelidium* and *Pterocladia* are similar morphologically, and their differentiation has long been problematic (see e.g. Santelices, 1974, unpubl. mscr.; Stewart, 1976; Akatsuka, 1986a; Rodriguez and Santelices, 1987, unpubl. mscr.). Rodrigueuz and Santelices (unpubl. mscr.) reviewed characters previously proposed for separating these genera. Currently, only the number of cystocarp locules (two in *Gelidium* and one in *Pterocladia*) can be used consistently to separate the genera. Okamura (1934) had suggested that rhizine position and medullary cell form are useful differentiating characters, generalizing that in *Gelidium* rhizines are distributed in the cortex and outer medulla and medullary cells are rounded and loosely packed, whereas in *Pterocladia* rhizines are distributed only in the inner medulla and medullary cells are angular and closely packed. Despite this distinction, Okamura noted some exceptions to these generic rhizine patterns. Others (i.e. Santelices, 1974; Stewart, 1976) have found these characters to be variable and inconsistent, and thus incapable of discriminating between *Gelidium* and *Pterocladia* (Rodriguez and Santelices, unpubl. mscr.). Stewart (1976) studying the California flora, proposed the presence or absence of basally incurved (geniculate) branches as a character to separate these two genera with geniculate branches present in *Gelidium* and absent in *Pterocladia*. However, this character

was found to be inconsistent when tested on more species of both genera (Rodriguez and Santelices, unpubl. mscr.).

Recently, Akatsuka (1981, 1986a, 1986c) proposed that surface cortical cell morphology, arrangement and orientation, in surface view and longitudinal section, are capable of differentiating Japanese species of *Gelidium* and *Pterocladia*. Akatsuka's criteria were found only to be consistent for the most basal parts of (upright) axes (Rodriguez and Santelices, unpubl. mscr.). Rodriguez and Santelices (1987, unpubl. mscr.) have proposed apical architecture characters that almost consistently separate *Gelidium* from *Pterocladia* (except *P. bulbosa* and possibly other *Pterocladia* species with acute apices). They suggested that apical and lateral initials are morphologically similar in *Pterocladia* but are different in *Gelidium* and that lateral initials may be in cortical indentations in *Pterocladia* but are never indented in *Gelidium*. These criteria were tested further on gelidioid species in British Columbia and northern Washington.

Rodriguez and Santelices (1987, unpubl. mscr.) implied that other features of apical architecture (lateral initials close to or far from the apical initial, apical initial protruding beyond or indented between adjacent cortical lobes, and lateral initials protruding from or indented between cortical lobes) also might be used for generic segregation of *Gelidium* and *Pterocladia*. While discussing the states of these characters in small groups of species of *Gelidium* and *Pterocladia* Rodriguez and Santelices (unpubl. mscr.) do not demonstrate how the characters may be used to vegetatively discriminate *Gelidium* and *Pterocladia*.

D. NEED FOR ADDITIONAL INFORMATION

Given the economic importance of Gelidiales as a high quality source of agars (Santelices, 1974, 1986), surprisingly little is known about the biology of members of the order. Many taxa have been described, but the morphological plasticity of species has caused great taxonomic confusion (e.g. Dixon, 1958), and few careful, comparative, monographic studies have been made. Gelidiaceae occupied a pivotal position in the definition of an auxiliary cell, yet there are still many aspects of pre- and post-fertilization development to be resolved, with few species having been studied in detail. Developmental studies of spermatogenesis and tetrasporogenesis, particularly ultrastructural, are needed to better understand developmental patterns that may help establish the position of Gelidiales with respect to other orders. The life history for members of the order has been presumed *Polysiphonia*-type and was recently determined from culture studies for only two species (Macler and West, 1987; van der Meer, pers. comm.); further confirmation is needed in other species, particularly the generic types from their type locality. The seaweed flora of British Columbia and northern Washington is being revised (Garbary *et al.*, 1980, 1982; Hawkes and Scagel, 1986a, 1986b; Lindstrom and Scagel, 1987), and this research on Gelidiales contributes to this goal.

CHAPTER II. MATERIALS

Collections of *Gelidium coulteri*, *G. purpurascens*, *G. vagum* and *Pterocladia caloglossoides* were made on the southern part of British Columbia and some of the San Juan Islands (Washington). In addition, herbarium specimens were examined from previous collections throughout the study area. Most of this study was carried out on freshly collected or 5% Formalin or acetic alcohol (3:1 ethanol:glacial acetic acid) liquid preserved plants. Herbarium collections were relied on for type specimens, for representatives from a taxon's range, and for comparisons of local species with specimens which do not occur in the study area.

A total of 56 field collections were made from 27 sites between Nov. 1983 and Aug. 1987 (Table 2). Collections were made intertidally and from the shallow subtidal using SCUBA. Some sites were selected based on previous reports of gelidiaceous algae (e.g. South, 1968; Scagel, 1973; Garbary *et al.*, 1984), or from herbarium records. Other sites were selected on the basis of a suitable habitat and substratum (e.g. bedrock, boulders). All collection sites (with the exception of the Barkley Sound locations) were chosen because of their relative accessibility to shore diving; a boat usually was not available.

Of the collection sites (Figs. 1-4), eleven sites were in Barkley Sound, west coast of Vancouver Island (Fig. 2), seven in the Strait of Juan de Fuca and Gulf of Georgia (Fig. 3), five on Hornby and Denman Islands, mid-way along the western side of the Strait of Georgia (Fig. 4), and four mid-way along the

eastern side of the Strait of Georgia on the mainland (Fig. 4).

Frequent collections were made at Orlebar Point, Gabriola Island (Table 2) in the Strait of Georgia, a site chosen for its accessibility (suitable for a day trip), good diving conditions (safe entry, some protection from winter storms and availability of accurate marine weather reports) and the abundance of three gelidiaceous species. *Gelidium* and *Pterocladia* populations were studied approximately every 6-8 weeks throughout the year. Collections were qualitative with presence/absence, growing condition, and reproductive state of the plants noted.

Herbarium specimens were obtained from AHFH, CANA, FHL, MICH, TCD, UC, US, USM and WTU (see Table 3 for abbreviations; Holmgren *et al*, 1978). After the AHFH loans used in this study were obtained, the AHFH collection was moved to LAM. A detailed list of all herbarium specimens studied is given in Appendix 1.

CHAPTER III. TAXONOMY AND NOMENCLATURE

A. INTRODUCTION

Since the first published records of Gelidiaceae in the study area (Setchell and Gardner, 1903), a total of nine species of *Gelidium* Lamouroux (1813: 128) and *Pterocladia* J. G. Agardh (1852: 482) have been reported. Setchell and Gardner (1903) reported *Gelidium amansii* Lamouroux (1813: 41) from Vancouver Island as have subsequent reports (Collins, 1913: 114; Kylin, 1925: 13 and Connell, 1928: 100). Scagel (1957: 142) interpreted all of these reports to be *G. crinale* (Turner) Lamouroux (1825: 191). [Kylin's (1925) report of *G. amansii* is a repeat of Setchell and Gardner's (1903) record, because he stated that he did not see *G. amansii* in his collections]. At least some early "*G. amansii*" records can probably be attributed to *G. purpurascens* Gardner (1927a: 275), a taxon recorded [by Scagel (1957: 141, as *G. amansii*] from the southern part of Vancouver Island (CANA 3843, CANA 4346, CANA 4349).

A second species, *Gelidium cartilagineum* (L.) Gaillon (1828: 15) was reported by Kylin (1925) from San Juan Island, Washington. Gardner (1927a: 280) described *G. cartilagineum* var. *robustum* to encompass the Californian plants previously referred to *G. cartilagineum*. Previous British Columbian and northern Washington records of *G. cartilagineum* were considered to be *G. cartilagineum* var. *robustum* by Scagel (1957). These large, robust plants were later reported by Hollenberg and Abbott (1965: 1179) as a distinct species, *G. robustum*.

Gelidium coulteri Harvey (1853: 117) was collected first from the southern part of Vancouver Island (as *G. crinale* in 1908, CANA 3474, Scagel, 1957). The identification of this species in the study area as *G. coulteri* first was made by Harlin (1969: 258) and Abbott (1970: 1) from Washington, and by Scagel (1973: 138) from British Columbia. Scagel (1957) first reported *G. crinale* in British Columbia and Washington (CAN 207, now CANA 3474, and V 1456 (V=herbarium of the British Columbia Provincial Museum). *Gelidium sinicola* Gardner (1927a: 278) was mentioned first in descriptive ecological works (Stephenson and Stephenson 1961a: 15 and 1961b: 229) and later as a taxonomic record (Norris and West, 1966: 176). *Gelidium pulchrum* Gardner (1927a: 279) has been recorded only once in an ecological study of zonation in the Strait of Georgia (Stephenson and Stephenson, 1961b: 229). Norris and Wynne (1968) reported *G. pusillum* Le Jolis (1863: 139) from Washington, but there are few (Widdowson, 1974; Scagel *et al.*, 1986) there are few subsequent reports of this taxon in the study area. Similarly, there are few records of *Pterocladia caloglossoides* Dawson (1953: 76), since it was reported by Norris and Hollenberg (1969: 116). The most recent addition to the gelidiaceous flora of British Columbia was *G. purpurascens* Gardner (1927a: 279) (Scagel, 1973; Waaland, 1973).

A larger number of gelidiaceous taxa occur in adjacent regions of the Pacific (Table 4). To the south, 11 species of *Gelidium*, six species of *Gelidiella* Feldmann *et* Hamel (1934: 529) and four species of *Pterocladia* are present in California, Pacific Mexico and the Gulf of California (Dawson, 1944, 1953; Stewart 1976; Stewart and Norris, 1981; Santelices and Stewart, 1985). From Pacific Panama, two species of *Gelidium* and one species of *Gelidiella* were

reported in an ecological study (Lubchenco *et al.*, 1984). Nine species of *Gelidium*, one *Gelidiella* and four *Pterocladia* are recorded from Pacific Colombia, Peru and Chile (Santelices and Stewart, 1985; Santelices and Montalva, 1983; Schnetter and Bula Meyer, 1982; Dawson *et al.*, 1964). From Hawaii, four species of *Gelidium*, four *Gelidiella* and four *Pterocladia* are currently recognized (Santelices, 1977; Santelices and Stewart, 1985). Numerous species of *Gelidium* and *Pterocladia*, plus species of *Beckerella*, *Acanthopeltis*, *Gelidiella*, *Onikusa* and *Yatabella* have been reported from the northwest Pacific. From Japan, 24 *Gelidium* species, two *Gelidiella* species, four *Pterocladia* species, two *Beckerella* species and a single species each of *Onikusa*, *Acanthopeltis* and *Yatabella* are reported (Akatsuka, 1986b). For China, eight *Gelidium* species, one *Gelidiella* species and two *Pterocladia* species are recorded (Bangmei *et al.*, 1983; Santelices and Stewart, 1985) some in common with Japan. Hommersand (1972) noted some floristic elements in common between northeast and northwest Pacific areas, suggesting possible floristic affinities of some Gelidiaceae between British Columbia and the northwestern Pacific.

At present, the distinction between *Gelidium* and *Pterocladia* can only be definitively determined by the number of locules in the cystocarp (Santelices, unpubl. mscr.). Several other vegetative features have been suggested for generic separation. Characters such as rhizine distribution (subcortical or outer medullary in *Gelidium* versus inner medullary in *Pterocladia* by Okamura, 1934), morphology of emergence of branches from the main axis (branches basally bent in *Gelidium* and unbent in *Pterocladia*, Stewart, 1968) and shape and orientation of cortical cells (Akatsuka, 1981, 1986a) have been found inconsistent and unreliable when

tested on larger numbers of taxa (Rodriguez and Santelices, unpubl. mscr.). Recently, Rodriguez and Santelices (1987, unpubl. mscr.) proposed shape and position of the apical and lateral initials to distinguish these two genera. They proposed that in *Pterocladia* apical and lateral initials are indistinguishable in shape and always found depressed below the level of outer cortical cells, whereas in *Gelidium*, the lateral initials are different in shape from apical initials and are never present in cortical depressions (or could be, in a few cases, in indistinct depressions). These characters were consistent for a large number of Pacific species, with the exception of *Pterocladia bulbosa* Loomis (1960: 7) (Rodriguez and Santelices, unpubl. mscr.). All of the features were tested in the species of *Gelidium* and *Pterocladia* in this study and are discussed under each taxon.

B. METHODS

Material was hand-sectioned, or sectioned to 10-20 μm on a Bailey Instruments Tissue-Freez freezing microtome, thick enough to determine relationships between cells as indicated by pit-connections and to trace files of cells. Transverse and longitudinal sections were stained using 1% aqueous aniline blue for 15-45 s, fixed with 10% HCl, washed with distilled water and mounted in 40% Karo clear syrup to which a small amount of Formalin and aniline blue stain had been added. Nuclei were observed by staining sections with Wittmann's hematoxylin (Wittmann, 1965) for one hour, followed by fixing with 45% acetic acid, and washing and mounting in either distilled water or in 40% Karo (with a few drops of Formalin added).

In some specimens, apices, tetrasporangial stichidia, male or female

gamete-producing branchlets and/or cystocarps were excised. Tissue was fixed in 2.5% glutaraldehyde in Sorensen's phosphate buffer (pH 7.2), washed in buffer, dehydrated through a graded methanol series, infiltrated through a graded series of JB4 catalysed solution A concentrations and embedded in activated JB4 methacrylate (Ruddell, 1967; Appendix 2). The JB4 blocks were sectioned on a Sorvall JB4 microtome. Other material was embedded in Spurr's low-viscosity epoxy resin (Spurr, 1969) and fixed following the recipe described under transmission electron microscope (TEM) fixation (Appendix 3). Blocks were sectioned with a glass knife on a Reichert OM U3 ultramicrotome at approximately $2.5\mu\text{m}$. Sections of embedded material were then stained with toluidine blue (pH 4.4) for 20 s, rinsed in tap water and mounted in 40% Karo clear syrup with Formalin and aniline blue stain added.

For each taxon, measurements giving maxima or minima (e.g. maximum height) are taken from all specimens studied. Axis width and branch angle measurements were made from 10 plants, selected haphazardly. For cortical cell, medullary cell and rhizine sizes, 20-50 measurements were made on 4-5 plants of each taxon from different localities for which fresh or Formalin-preserved specimens were available. Carpogonial size was taken from 4-5 measurements on 4-5 plants; cystocarp diameter was from 10 measurements on 1-2 plants; carposporangial size was from 20 measurements on 4-5 plants. Twenty measurements of spermatium diameter were made on 2 plants. Tetrasporangial stichidium length and diameter of tetraspore release holes was based on 16-20 measurements of 4 plants; tetrasporangial size was taken from 30-40 measurements of 3-4 plants, except for *Pterocladia caloglossoides* where sporangial size was from 15 measurements on 3

plants.

The "!" notation, given with the information on type specimen for the species in this study, indicates that I have seen the type(s), and *n.v.* (*non vide*) indicates that I have not seen the specimen(s).

Rhizine ultrastructure was observed in sterile (from Orlebar Point, Gabriola Island) and spermatangial thalli (from male gametophytes cultured from tetraspores of whalebone Bay, Gabriola Island) of *Gelidium purpurascens*, and in the spermatangial part of *G. vagum* gametophytes from Tribune Bay, Hornby Island. Longitudinal sections of axes 1 mm long, from 3-5 mm proximal to the branch apex (for young rhizines) and from 2-3 cm proximal to the branch apex (for older rhizines), were prepared for TEM study.

C. TAXA PRESENT IN BRITISH COLUMBIA AND NORTHERN WASHINGTON

Four gelidiaceous taxa in the study area are morphologically distinct and reproductive characters confirm their identities (Fig. 5). The Gelidiales in the local flora are *Gelidium coulteri*, *G. purpurascens*, *G. vagum* Okamura (1934: 58) and *Pterocladia caloglossoides*. Specimens upon which records of *G. robustum*, *G. crinale*, *G. sinicola* and *G. pusillum* were based were re-examined and found to be misidentifications of the four taxa reported herein. The report of *G. pulchrum* Gardner (1927a: 279) (Stephenson and Stephenson, 1961b) is unsubstantiated.

1. *Gelidium coulteri* Harvey

1853: 117

Synonym: *G. undulatum* Loomis, 1960: 4, pl. 6, fig. 1-4, n.v.

Lectotype: TCD, collected by Dr. Coulter. Selection of the lectotype was by an unknown person, and it is not known if the designation was ever published (Parnell, pers. comm. 1988). Harvey (1853) described a tetrasporangial plant, so the lectotype specimen should be a tetrasporophyte, n.v.

Type locality: California, U.S.A.

Isotypes: TCD!, AHFH 53929!

a. Habitat and Habit

This is the only local *Gelidium* species that is restricted to the intertidal, occurring epilithically at relatively protected sites from the mid-littoral to the 0 m tidal level (Canadian chart datum). Plants are soft and non-cartilaginous, although short plants can be slightly stiff. They are brown to reddish-brown when fresh and dry to brown-black. The growth form is a turf, small thalli (to 2 cm tall) are dense, whereas taller thalli (to 5 cm tall) are less dense. Plants from protected sites tend to form taller, looser turfs. Upright axes arise from branched, creeping axes, and may divide to form 2-3 main axes, up to 5 cm tall, but more commonly 1-3 cm tall.

Branching is sparse and usually only to two orders, though occasionally some

third order branches may be present (Fig. 6). Branch angles are wide, 45-60°, on widely spaced, irregularly to suboppositely positioned branches. Many branches are short and determinate, giving axes a narrow outline. Some short plants appear densely branched but to a higher order, since branches are more closely spaced together with relatively fewer short proliferous-like branches. Axes are terete and usually narrow, to 0.5 mm wide although occasionally axes may be up to a maximum of 1 mm and compressed. This wider, compressed form was less common. Each branch order is narrower than the one it arises from. Branches taper abruptly at the apex, but branch bases are untapered and unstricted.

Creeping, terete axes of smaller diameter than uprights, that produce occasional, scattered, pads attach the thalli to the substratum. Attachment pads are colourless, formed of elongated cortical and medullary cell extensions amassed together, and somewhat spreading on contact with the substratum (Fig. 8). Attachment pads are absent in upright axes.

b. Vegetative Anatomy

Thallus construction is uniaxial, with a domed apical cell (Fig. 7) producing a subapical cell that cuts off two periaxial cells. In transverse section, cortical and medullary layers are apparent (Figs. 9, 10). The outer cortex is two to three cell layers and is composed of cells of approximately the same size. Outer cortical cells of uprights are isodiametric, 3-8 μm long and 3-8 μm wide, those of the creeping axes are larger and more oval, 6-16 μm long and 4-9 μm wide.

In surface view, cortical cells of both upright and creeping axes (viewed approximately halfway between plant apex and base) are randomly oriented and equidistant, showing no pattern or groups (e.g. pairs or tetrads) (Fig. 11). Secondary pit-connections are absent between the outermost cortical cells, but are present between second and third cortical cell layers of the cortex. The inner cortex, of 1-2 cells, grade to the medulla (Figs. 9, 10), and become progressively larger and more elongate inward. The medulla occupies approximately 75% of the axis radius (Fig. 9), with elongate, thick walled cells, 28-228 μm long and 10-21 μm wide, that are widely spaced, and have abundant secondary pit-connections to adjacent cells (Fig. 10). Intercellular spaces are packed with rhizines (Figs. 9, 10), which are also present in the inner cortex, being sparse in young axes but abundant in older ones. Unicellular and thick walled, rhizines are small diameter (3-4 μm) and are very elongate (at least 500-700 μm long) (Fig. 10), and also present in creeping axes.

c. Reproductive Anatomy

Tetrasporangia are restricted usually to determinate fertile branches (stichidia). Stichidia in *Gelidium coulteri* are elongate, (proportionately longer and narrower than in other local *Gelidium* species), 0.7-1.9 mm long and 0.2-0.4 mm wide, and generally appear somewhat like proliferous branchlets (Fig. 12). The stichidia may themselves bear a few short branchlets (Fig. 13), arranged irregularly alternate to subopposite on higher branch orders or on distal parts of major axes (Fig. 12). Tetrasporangia are scattered irregularly on stichidia (Fig. 14), with a tendency for older tetrasporangia to be in the more basal parts of stichidia.

Older stichidia produce additional tetrasporangia following release of those formed first (Fig. 16). Developing tetrasporangia first are recognizable 90-300 μm behind the apex, are cut off from a cortical cell 3-4 cells below the surface. The pit-connection to the bearing cell is basal initially and becomes lateral as the sporangium increases its size with much inward expansion (Fig. 15). Tetrasporangia divide successively [i.e. cytokinesis of the first (transverse) division is completed before the second cytokinesis is initiated], to form four cruciately arranged tetraspores (Figs. 15, 17). At maturity, tetrasporangia are oval and deeply embedded in the cortex, but with their outer ends close to the thallus surface (Fig. 17). Mature tetrasporangia are 26-41 μm long and 16-31 μm wide, with adjacent cortical cells usually elongated and curved around them.

Gametophytes of *Gelidium coulteri* have not been collected from the field in the study area, but male gametophytes have been grown in culture from released tetraspores. Female gametophytes were not detected in cultures, possibly because of low numbers of germinated sporelings. Gametophytes are assumed to be dioecious because male gametophytes only produced male gametes.

Spermatangia are borne in superficial sori near the bases of indeterminate branches. Strongly fertile male gametophytes produce spermatangia along the branch to within a few millimeters of the apex. At low magnification, spermatangial areas appear pale pink (vs. brownish red where not fertile) because spermatangia are not pigmented. The fertile area appears slightly thicker because the wall is ruptured by spermatangial release, and an abundance of mucilage is present (Fig. 18). Spermatangial mother cells differentiate from cortical cells and

are half the diameter of vegetative cortical cells. Each spermatangial mother cell cuts off a single spermatangium by a transverse (periclinal) division (Figs. 19, 20). Spermatia released from spermatangia are small, 2.1-3.1 μm at widest diameter, and hemispherical to spherical in shape.

d. Discussion

The intertidal habitat, few orders of branches, narrow branch outline, spindle-shaped tetrasporangial stichidia, turf-like growth habit and dark colour distinguish *Gelidium coulteri* from other gelidiaceous taxa in the study area. Particularly diagnostic are the abundant, short, simple branchlets. I found that British Columbia plants of *G. coulteri* correspond well with *G. coulteri* in California (Figs. 21-24). A comparison of type material (lectotype and isotype in TCD, Figs. 22, 24; isotype AHFH 53929, Fig. 23) from Monterey Bay, California with British Columbia plants indicates that plants from the two localities are conspecific. They have similar branching patterns, but Californian plants are more branched and appear bushier. The fertility (tetrasporangial) of some of the Californian plants also adds another order of (stichidial) branches, contributing to their bushy appearance.

Cystocarps of *Gelidium coulteri* are known from California, but evidence for the placement of this species in *Gelidium* is supported additionally by features of apical and lateral branch initials. Apical initials are large and domed, protruding beyond adjacent cortical cells and form acute branch apices (Fig. 7). Lateral initials differ from apical initials, being inverted conical, but are similar in

position to apical cells as both initials are not in depressions of the cortex. These observations agree with Rodriguez and Santelices' (1987, unpubl. mscr,) proposed use of vegetative features to characterize *Gelidium*. disagreement with their *Pterocladia* characters.

A number of reports pertaining to collections of *Gelidium coulteri* from British Columbia and several misidentified herbarium specimens of *G. coulteri* (in UBC), show that this species has been confused with other taxa. At times *G. coulteri* has been referred to erroneously as "*G. crinale*" (e.g. Scagel, 1957 - CANA 3474 (formerly as CAN 207); Scagel 1973 - UBC A40467, UBC A40468, UBC A40469, UBC A40470, UBC A31363, UBC A31426, UBC A37402, UBC A53975, UBC A60236, UBC A60254, UBC A60268, UBC A64934), as "*G. pusillum*" (e.g. UBC A24837, UBC A28351; FHL 3055), as "*G. sinicola*" (e.g. UBC A1449, UBC A14403, UBC A36545), as "*G. robustum*" (e.g. South, 1968 vouchers UBC A28645, UBC A29575, UBC A36198, UBC A36199), as "*Caulacanthus ustulatus*" (UBC A31363) and as "*Endocladia muricata*" (UBC A12298, UBC A13259, UBC A19739). In a few cases (UBC A30401, UBC A312511, UBC A37605), *Pterocladia caloglossoides* has been misidentified as "*G. coulteri*". These other taxa (*G. crinale*, *G. pusillum*, *G. sinicola* and *P. caloglossoides*) are generally poorly understood, and probably accounting for their confusion with *G. coulteri*.

The report of *Gelidium sinicola* by Stephenson and Stephenson (1961b) from the area of Nanaimo, British Columbia may represent a mistaken identification of *G. coulteri*. Unfortunately, the authors gave no voucher numbers, description or

figures of the reported taxon, and therefore the plants' identity cannot be confirmed. Collections from the area (Orlebar Point, False Narrows, Lock Bay and Davisons Beach, all on Gabriola Island, near Nanaimo), however, show that *G. coulteri* is common. Stephenson and Stephenson were listing plants in the infralittoral zone, and they referred to *G. sinicola* as a short, moss-like alga. They also reported the presence of *G. pulchrum*, a larger plant with a wiry texture (probably *G. purpurascens*, see discussion later in this chapter). This allusion to a turf-like growth habit and short stature suggests that *G. coulteri* is probably the taxon that Stephenson and Stephenson (1961b) were calling *G. sinicola*.

e. Seasonality and Distribution

Gelidium coulteri is perennial, persisting as short uprights when not growing actively. Year round observations of *G. coulteri* were made at Orlebar Point, Gabriola Island (Fig. 3). Plants began showing growth from regenerated apices in early spring (March) and continued growing throughout the summer. They became fertile (tetrasporic) in August, remained fertile until November, but those few plants that were fertile did not produce tetrasporangia abundantly. Growth slowed in November and December, indicated by increasing overgrowth of apices by diatoms. Plants ceased growth in January and axes were eroded back to shorter lengths. Apices could not be detected and branch ends appeared truncated and broken.

Gelidium coulteri is common throughout most of the study area and was recorded

from almost all of my collection sites that had *Gelidium* or *Pterocladia*. Its range extends north on both the open Pacific and Strait of Georgia shores of Vancouver Island, and on the British Columbia mainland to the Queen Charlotte Islands. The most northerly collection is from Pincer Island, British Columbia (52°11'N latitude). *Gelidium coulteri* ranges south, through Oregon (Doty, 1947) and California to Punta Pequeña, (Pacific) Baja California del Norte, Mexico (Dawson, 1953).

2. *Gelidium purpurascens* Gardner

1927a: 275

Synonyms: *Gelidium contortum* Loomis, 1960: 4, n.v.

Gelidium densum Gardner, 1927a: 278, n.v.

Gelidium distichum Loomis, 1949: 2, n.v.

Gelidium gardneri Loomis, 1960: 5, n.v.

Gelidium papenfussii Loomis, 1949: 1, n.v.

Gelidium polystichum Gardner, 1927a: 276, n.v.

Gelidium pulchrum Gardner, 1927a: 279, n.v.

Gelidium ramuliferum Gardner, 1927a: 279, n.v.

Gelidium setchellii Gardner, 1927a: 275, n.v.

Holotype: UC 93572 cystocarpic!

Type locality: Moss Beach, San Mateo County, California, U.S.A.

a. Habitat and Habit

Gelidium purpurascens is one of the more conspicuous gelidiaceous species in the study area. It occurs epilithically in protected sites in the shallow sublittoral from 0-12 m depths, but is most abundant and vigorous at 0-5 m depths. *Gelidium purpurascens* may be present at localities where there is much siltation, but it is absent from kelp and seagrass beds. This species is often one of the dominant seaweeds where it occurs.

Plants are robust, cartilaginous, stiff (they are stiff enough to support their branches when out of the water), and dark red to maroon and dry to blackish-red. Plants grow as individuals or in small clumps and do not form a turf. Individuals may be much-branched and "brush-like" if older, or pyramidal and flat when younger (Fig. 25). Most of the biomass is in upright axes, which are attached to the substratum by a smaller system of creeping axes. Usually a single upright axis arises from creeping axes, but the upright may divide to produce 2-4 main axes that can grow to 15 cm tall.

Plants are well-branched with up to four orders of branches (Figs. 25, 27). Branches are distichous to irregularly arranged, being subopposite to alternate in different parts of the plant or even along the same axis. Branches are indeterminate, such that older branches are longer and more branched than younger ones. This gives a pyramidal aspect to at least well-branched sectors of the plant. A divergent branching angle of 30-45° also contributes to the triangular outline of plants. Upper portions of plants appear best developed,

whereas lower portions often are less well-branched, possibly because of shading or damage from grazers or abrasion. Proliferous branches may develop, but these are not common or abundant. Axes are terete to compressed. Main axes may be 1 mm wide, and progressively higher orders of branches are successively narrower with the highest order branches to 250 μm wide. Branch apices are acute to rounded, with bases that are not tapered or constricted.

Creeping axes are terete, narrower than major and second order upright axes, less deeply pigmented and sparsely and irregularly branched (Fig. 27). Attachment of creeping axes to the substratum is by occasional attachment pads (Fig. 33) that are colourless and formed from elongated cortical cells amassed together into a solid structure (Fig. 34). They are found only on creeping axes (there is no secondary attachment of upright axes).

b. Vegetative Anatomy

The thallus is uniaxial with a domed apical cell, not obvious at low magnification but easily visible in longitudinal or saggital sections (Fig. 28). Each subapical cell cuts off two, laterally opposite, periaxial cells. In transverse sections a pigmented cortex and unpigmented medulla can be distinguished with the inner cortex grading into the medulla without an abrupt transition (Figs. 26, 29). Surface cortical cells are smallest (4-9 μm long and 2-7 μm wide), and inner cortical cells are progressively larger (Figs. 29, 30). Outer cortical cells are isodiametric to oval with their longer axes, if detectable, randomly oriented (Fig. 31). Inner cortical cells are oval to short cylindrical, their length being approximately twice

their width (Fig. 30). Secondary pit-connections are abundant between inner cortical cells but are absent from the outer two cortical layers. Medullary cells are cylindrical, 18-107 μm long and 10-25 μm wide with innermost cells longest (Fig. 30), and have abundant secondary pit-connections (Fig. 32).

Rhizines are abundant in the inner cortex and outer medulla, but in decreased abundance in the central medulla (Fig. 26). They are present in creeping axes in the same position and abundance as in uprights. Rhizines are unicellular, narrow (3-4 μm wide), very thick walled and unbranched (Figs 29, 30). They are cut off from the inner proximal corner of inner cortical cells 280-325 μm and develop proximal to the apex as a small protuberance, growing basipetally between inner cortical and medullary cells to at least 2-3 times the length of the longer medullary cells (i.e. at least 300 μm long).

The cell wall is the rhizine's most conspicuous feature. Its radius (1000-1500 nm) is greater than or equal to the diameter of the protoplast, and the wall is thicker in older cells (Fig. 36) than in younger ones (Fig. 35). Fibrils of the wall are interwoven, but generally run parallel to the long axis of the cell (Fig. 39) and are loosest near the plasma membrane (Fig. 35). The ground matrix between fibrils is electron transparent. The metabolism of young rhizines appears to be directed toward wall deposition. The plasma membrane has a convoluted profile (Fig. 35). Mitochondria are prominent and closely associated with the forming face of dictyosomes (Fig. 39). Dictyosomes are abundant and large, 500-1000 nm wide and 600-800 nm tall (Fig. 39), their mature vesicles containing fibrillar material similar in diameter and electron density to the cell

wall fibrils (Fig. 39). However, there is no evidence of vesicles with fibrillar contents secreting to the wall, even in cells showing numerous secretory vesicles. Another form of vesicle (Figs. 37, 39), containing spherical and tubular bodies and resembling multivesicular bodies or lomasomes (Scott and Dixon, 1973b), is present. The origin of these vesicles is unclear, but they do not appear to be directly dictyosome-derived. There is evidence of many of these vesicles fusing with the plasma membrane and releasing their contents to the wall (Figs. 37, 39). Outside and immediately adjacent to the plasma membrane are many tubular body fragments that are not present in older walls, farther away from the cytoplasm. The tubular bodies must either degenerate or become dispersed, forming new wall. The abundance of both types of vesicles suggests the maturation of the fibrillar contents into tubular bodies and their subsequent release to the cell wall. In young rhizines, near their point of initiation from a subcortical cell, proplastids are present (Fig. 38, arrow) with only an inner encircling thylakoid, a few plastoglobuli and areas of DNA. These proplastids, included in the rhizine cytoplasm at the time of division of the subcortical cell, do not replicate; they are seen only occasionally and are absent from older or more distant parts of the rhizine.

There is much less secretion and wall deposition activity in old rhizines. The cytoplasm is vesiculate and vacuolate (Fig. 40), many of the vesicle membranes look degenerate, mitochondria are few and the cytoplasm is thin and sparse. The plasma membrane profile is smooth (Fig. 36) and not convoluted as in more active cells.

c. Reproductive Anatomy

Tetrasporangia are located in determinate, stichidial branchlets of upright axes (Fig. 41) that are short and clavate, compressed, and approximately twice as wide as vegetative branchlets of the same order or one order lower. Stichidia usually form on distal parts of plants or axes and impose an additional order of branching over the vegetative branching pattern. Thus tetrasporangial plants appear denser and more branched.

Tetrasporangia are scattered over the stichidial surface, but are absent from the narrow sterile margin (Fig. 42). Sporangia are not oriented in rows, and different ages are interspersed due to the continuous production of tetrasporangia (Fig. 42) after mature ones are released. Similar ages of sporangia are approximately equidistant. Tetrasporangia are first distinguishable 30-60 μm proximal to the apex (Fig. 43). The pit-connection to the subtending cortical cell is initially basal (arrowheads in Fig. 43), but later becomes lateral as the tetrasporangium expands deeper into the branch. Tetrasporangia are cruciately divided with the first division periclinal and the second (in each half) anticlinal (Fig. 44). At maturity tetrasporangia are 40-81 μm long and 20-49 μm wide. Tetraspores are pyramidal on release because of mutual compression in the sporangium, but soon round up. The cortex of the tetrasporangial part of the stichidium is thicker than the vegetative axes and occupies 2/3 of the axis radius. Inner cortical cells adjacent to the widest part of the tetrasporangium are elongated.

Gelidium purpurascens is dioecious, and male and female gametophytes occur in

low numbers in field collections. Gametophytes are isomorphic with tetrasporophytes. Carpogonia and cystocarps occur terminally on highest order branches or on short, side branchlets of upright axes (Figs. 45, 48). These branchlets have the capacity to continue growing, but do not if reproduction is successful. Occasionally a second fertile area occurs when apical growth continues beyond the first set of carpogonia (Fig. 48). The apex of the carpogonial stichidium of *G. purpurascens* is acute, with the apical cell not recessed into an apical notch as reported by Hommersand and Fredericq (1988) for *G. pteridifolium* from South Africa.

Numerous (20-32) carpogonia develop in the fertile area, and are initiated five to seven axial cells proximal to the apex (Fig. 47). The apex continues to grow, and later in development the cystocarp appears 1-3 mm proximal to the apex. Carpogonia are 13-29 μm long and 3.5-5.5 μm wide. Sizes are approximate as measurements did not account for the curvature of cells. Carpogonia differentiate in third order filaments above and below the second order plate (i.e. a plate of second order filaments connected by secondary pit-connections), and their trichogynes project to both surfaces of the branchlet (Fig. 47). Carpogonia are intercalary in vegetative filaments and have two pit-connections (Fig. 46). The carpogonium is the second cell in a third order filament cut off from a cell of the second order plate, close to the axial filament. The upper pit-connection of the carpogonium is to a vegetative cell (Fig. 46) that produces higher order vegetative branches. In non-fertile axes, the cell in the position of the carpogonium has a second upper pit-connection (three pit-connections in total) to another branched vegetative chain. Thus, the carpogonium replaces a vegetative

chain whose outermost cells contribute to a small patch of thallus surface. The absence of surface cortical cells, which have been replaced by the carpogonium, leaves a small gap or "hole" in the thallus surface. The confluence of adjoining "holes", resulting from a double row of carpogonia, creates a medial furrow in the upper and lower surface of the female branchlet. Trichogynes protrude through this gap.

Carpogonia farther back from the apex are sessile, cut off from the subtending cell by a distinctive concave wall. It was determined (see Chapter 5) that intercalary carpogonia are functional and may be fertilized. When they are past fertility, the concave wall cuts off the distal part of the carpogonium, which then degenerates while the "supporting" cell remains functional.

Upon fertilization, nutritive filaments composed of short, curving chains of small, densely staining, globular cells, proliferate from basal cells of third order filaments and curve towards and around second order plate cells. The fertilized carpogonium forms a large, multilobed, multinucleate fusion cell by fusing with surrounding cortical cells. The fusion cell cuts off gonimoblast initials that form a branching, filamentous gonimoblast and that produce uninucleate carposporangia terminally and laterally (Fig. 51). A more detailed description of post-fertilization development is given later in Chapter 5.

Mature cystocarps of *Gelidium purpurascens*, 420-560 μm in diameter, are bilocular with one ostiole per locule (Fig. 50). They are spherical, protruding from both surfaces of the branch and are wider than the sterile portion of the branch

(Fig. 50). The carposporophyte is centered around the second order plate (Fig. 50) of gametophyte cells. The cystocarp cortex is the same thickness as the vegetative cortex, but it is composed of more cell layers. Cystocarp cortical cells are smaller and somewhat more tightly packed than in the vegetative cortex. Mature cystocarps of *G. purpurascens* have characteristic filaments between second order plate cells and the cortex (Figs. 49, 50). These filaments are third order files of cells that arise from cells of the second order plate, contribute to the cortex and extend to the surface. As the cystocarp cavity expands and the cortex is pushed away from the second order plate, third order filaments stretch but do not break and are thus intact in the mature cystocarp. There is no evidence to suggest a nutritive function for these stretched filaments; they are simply part of the vegetative construction of the thallus over which cystocarp morphology is imposed. Filaments of a similar appearance have been reported as "nutritive filaments" in other genera, e.g. *Gracilaria* (Dawson, 1949), but they are not homologous to "nutritive filaments" of *Gracilaria*.

Carposporangia are 20-39 μm long and 12-22 μm wide, ovate to obpyriform, uninucleate, pigmented, have a large, stellate chloroplast and are formed singly, i.e. not in chains (Fig. 51). They have a thick coating of mucilage or polysaccharide that fills the cystocarp cavity. Carpospores are released through an ostiole in the pericarp wall.

Spermatangia are produced in superficial sori on short stichidial branchlets of upright axes (Fig. 54), similar in position and arrangement (Fig. 52) to tetrasporangial stichidia. In living plants at low magnification the fertile area has

a colourless halo (Fig. 54) that corresponds to the spermatangial mother cell wall ruptured by gamete release, partially empty of spermatia and surrounded with large amounts of mucilage. Surface cortical cells 200-300 μm proximal to the apex are transformed by one to two longitudinal divisions into two to four spermatangial mother cells replacing a single outer cortical cell. Each spermatangial mother cell cuts off a single spermatangium by a characteristic transverse (periclinal) division (Fig. 56). As the spermatangium develops, an enlarging spermatangial vacuole appears basally as a clear area at high magnification. A single, conical spermatium, 3.1-5.2 μm diameter, is released through a narrow channel in the thickened wall (Figs. 55, 56), and rounds up into a spherical or short oval shape (Fig. 53).

d. Discussion

The bilocular cystocarp of this species dictates generic placement in *Gelidium*, as opposed to *Pterocladia*. Rodriguez and Santelices' (1987, unpubl. mscr.) proposed that *Gelidium* could be separated from *Pterocladia* using vegetative characters, of which two have been investigated here: 1) apical and lateral initials are morphologically different in *Gelidium*, whereas they are similar in *Pterocladia*, and 2) lateral initials never are found in indentations of the cortex in *Gelidium* whereas they are in *Pterocladia*. Apical architecture of *G. purpurascens* plants from the study area and from California agree with this suggested pattern: lateral initials are not in depressions and they are inverted conical in shape (Fig. 58), whereas the apical initial is domed and hemispherical (Fig. 57). Rodriguez and Santelices (unpubl. mscr.) also observed that the cortical cell pattern of the

basal 1-2 cm of upright axes could be used to discriminate between *Gelidium* and *Pterocladia*, but the cortical cell pattern was not consistent elsewhere on the axes as proposed by Akatsuka (1981, 1986a). In surface view, basal cortical cells of *G. purpurascens* are isodiametric, equidistant and are not aligned parallel to the axis (Fig. 60), in agreement with Rodriguez and Santelices' (unpubl. mscr.) characterization of *Gelidium*. Localized in small patches at the very base of uprights, elongate, elliptical, cortical cells have their long axes perpendicular to the branch axis (Fig. 61). This is unlike the pattern noted for both *Gelidium* (isodiametric and no alignment) and *Pterocladia* (elliptical and parallel to branch axis) (Rodriguez and Santelices, unpubl. mscr.), but this pattern is not universal or widespread on a branch.

The British Columbian entity called *Gelidium purpurascens* corresponds well with the holotype of *G. purpurascens* (UC 93572) (Figs. 62, 63) and other Californian material belonging to this species. Diagnostic characters given by Gardner (1927a) are that *G. purpurascens* (Figs. 62, 63) is tall, but not as large and coarse as *G. robustum* (Hollenberg and Abbott, 1965); well branched, including lower parts of axes; has compressed, basally constricted and geniculate pinnate branches to four or five orders; produces initially single cystocarps on fertile branchlets and later exhibits proximal, lateral, cystocarpic branchlets. Stewart (1976) modified the description to include geniculate or non-geniculate plants, with or without abundant basal branching and did not specify basal branch constriction as a diagnostic character. Plants collected in the study area fit the redefined description, particularly as branches of *G. purpurascens* are not basally constricted when compared to the marked basal constriction of higher order branches of *G.*

robustum.

Stewart (1976) generally described *Gelidium purpurascens* as large and similar in branching pattern to *G. robustum*, but not as coarse or tall as the latter species. A comparison of the holotype (UC 93572, Figs. 62, 63) and of specimens of *G. purpurascens* from the study site, with the holotype of *G. robustum* (UC 294572) (Fig. 138) showed that *G. purpurascens* and *G. robustum* are different morphologically. Although both species show the same number of branch orders, *G. purpurascens* is more proliferous and more densely branched. In *G. purpurascens* there are often several dominant axes that may divide into equivalent axes, whereas *G. robustum* has a single main axis obvious throughout the length of the plant. Transverse sections of vegetative branches of *G. purpurascens* show that the subcortex may be thick but that ordered rows of cells are not evident; similar sections of *G. robustum* branches also show a thick subcortex, but it is ordered into palisade-like rows. Tetrasporangial stichidia also differ between these species; stichidia of *G. purpurascens* have a narrow, almost inconspicuous, sterile margin, whereas stichidia of *G. robustum* have a wide sterile margin. Thus, despite some similarity in branching pattern, *G. purpurascens* can be distinguished morphologically and anatomically from *G. robustum* (Table 5).

Some of the earliest *Gelidium* collections from British Columbia are of *G. purpurascens* (CANA 3473, CANA 3740 (Fig. 59), CANA 3843, CANA 4346, CANA 4349, UBC A1952). These were called *G. cartilagineum* var. *robustum* (Scagel, 1957), and later *G. robustum* when Hollenberg and Abbott (1965)

elevated the variety to species status. Wherever possible, subsequent reports of *G. purpurascens* in the study area (Scagel, 1973; Foreman, 1977; Lindstrom and Foreman, 1978; Pueschel and Cole, 1982) have been confirmed. Publications that reported "*G. robustum*" in the study area (Scagel, 1967; South, 1968, record from Twin Beaches, Gabriola Island; Scagel, 1973), cited voucher herbarium specimens or illustrations, correspond not to *G. robustum*, but to *G. purpurascens*.

e. Seasonality and Distribution

Gelidium purpurascens is perennial, growing from April through October, but only persists as uprights with little growth (demonstrated by heavy diatom epiphytism) over winter months. Tetrasporophytes are fertile from February to December, most abundantly in May to October. Female gametophytes bear carpogonia in August and November collections, and male gametophytes are fertile in June, July and November.

Gelidium purpursacens is widespread throughout the study area and found along the British Columbia mainland, Queen Charlotte Islands, Hornby Island and Denman Island, Strait of Georgia, Gulf Islands, Strait of Juan de Fuca and along the length of the west coast of Vancouver Island. It is abundant at sites where it occurs and is present at all of my collection sites where any other species of *Gelidium* and/or *Pterocladia* are found.

Tetrasporophytes are fertile throughout the study area but fertile gametophytes are rare. Female gametophytes have been collected only from Diana Island and

Geer Islets, both in Barkley Sound (Fig. 2), and male plants have been identified only from Nootka Sound, Vancouver Island (Fig. 52), Esteban Point, Vancouver Island and Barkley Sound.

Gelidium purpurascens is distributed from Baja California del Norte (Stewart, 1976), to southeast Alaska (UBC A69432, UBC A69433). Its morphology is plastic, although in British Columbia it is distinctly different from other local species. In some environments plants are much less robust, paler and branches become long and flexuous, especially near apices, appearing similar to *G. vagum* Okamura (1934). The two species are still distinguishable by the cartilaginous texture of *G. purpurascens* (vs. lax and soft in *G. vagum*), the terete to compressed axes of *G. purpurascens* (vs. compressed to flattened in *G. vagum*), the narrow sterile margin of tetrasporangial stichidia in *G. purpurascens* (a sterile margin is absent in *G. vagum*), dioecious gametophytes of *G. purpurascens* (vs. monoecious gametophytes in *G. vagum*), and the presence of stretched third order filaments in mature cystocarps of *G. purpurascens* (these filaments are broken in *G. vagum* cystocarps).

3. *Gelidium vagum* Okamura

1934: 58

Type: A holotype was not designated by Okamura (1934) in the protologue.

Syntype specimens are in MAK (Akatsuka, pers. comm. 1987) but not available for loan. It is not known if Okamura designated a holotype, but no lectotype has been published.

Type locality: Not specified, although the distribution in Japan (around Honshu and southern Hokkaido) was described (Okamura, 1934).

a. Habitat and Habit

Gelidium vagum usually grows in the shallow subtidal from 0-13 m depths as well as in very low intertidal pools. Wherever plants or parts of plants have been exposed to air they are bleached and dead. This species is most abundant in the shallow subtidal, 0-4 m, where it may be a dominant, but it is not a dominant in populations of deeper water. *Gelidium vagum* grows epilithically on vertical walls and tops and sides of boulders, occurring in somewhat silted (e.g. Tribune Bay, Hornby Island) to protected localities (e.g. Denman Island and Ford Cove, Hornby Island) with a moderate amount of water motion. Plants are soft and lax and do not remain upright when exposed in air as does the more rigid *G. purpurascens*. They range from red to yellowish-red (deeper plants), and dry to brownish red, and grow as individuals, not as a turf, even though several upright axes may arise from the same creeping basal axis. Plants may be up to 10 cm tall, but are usually shorter (Figs. 64, 65), and can be fertile (tetrasporic or gametophytic) in the field at 1 cm tall.

Gelidium vagum shows considerable morphological variation in growth form, from thin and sparsely branched, to wide, robust and well branched. Generally, deeper-growing plants are thinner, lighter in colour, smaller and less branched. Plants are branched to the fourth order, and occasionally well-developed plants have fifth order branches (Fig. 64). Branching is divergent with a branch angle

of 45-60° for all ranks of branching. One to three main axes usually are distinguishable, although in some plants the main axis divides subdichotomously distally, and neither branch can be recognized as derivative. Branching is distichous and irregularly alternate to subopposite, and successive branches are well separated (approximately 1-2 mm apart). Shallow-growing plants may be quite bushy, whereas deeper plants are sparsely branched. Axes vary from compressed to flattened, with the most robust plants being conspicuously flattened, especially at branch points, and smaller, less robust plants terete to compressed. Main axes and lower order branches vary in width from 0.2-2.5 mm. Branch bases often are narrowest (but never constricted), with the branch axes widening gradually to the widest point, about 1-2 cm behind the apex. Higher order branches are usually progressively narrower and constricted basally, but may taper abruptly to acute apices, the distal 1 mm of which appears whitish or unpigmented in fresh material. Occasionally, ultimate branches become long and whip-like (Fig. 66). Higher order axes branch in the same way as main axes.

Creeping axes are pigmented, irregularly branched, terete, narrower than most upright branches and bear attachment pads. Attachment pads are unpigmented, scattered irregularly along prostrate axes formed from the confluence of elongated cortical cells, and spread out at the point of contact with the substratum.

b. Vegetative Anatomy

Thallus construction is uniaxial, with a conspicuous, domed apical cell domed (Fig. 68). Cells of the axial row each cut off laterally two periaxial cells (Fig. 68). In transverse section cortical and medullary layers are distinctive (Figs. 67, 71),

with a smooth and rapid transition between inner cortex and outer medulla. Outer cortical cells are isodiametric to slightly elongate, 4.0-11.3 μm long and 3.0-6.2 μm wide, with their longest axes irregularly oriented in the periclinal plane and equidistant (i.e. not associated into clusters) (Figs. 69, 70). However, near the apex, where cell divisions are occurring, the longest axes of outer cortical cells are in the anticlinal plane (Fig. 68). Secondary pit-connections are absent between cells of the outer two cortical cell layers, but are abundant between inner cortical cells. Inner cortical cells are larger and more elongate (16-60 μm long, and 12-29 μm wide) than outer cortical, and short and cylindrical with their long axis at approximately 30-45° to the branch axis. Medullary filaments are oriented periclinally and cortical filaments are anticlinal, so that the transition part of filaments of the inner cortex and outer medulla are oriented at oblique angles as noted by Akatsuka for *G. vagum* in Japan (1981, 1986a).

Medullary cells are larger (75-290 μm long and 10-29 μm wide) than inner cortical cells, with the longest cells deepest in the medulla (Fig. 72). They are cylindrical, unpigmented, thick-walled and have abundant secondary pit-connections (Fig. 68).

Rhizines are intercalary between inner cortical and medullary cells (Figs. 67, 71) and increase in abundance farther behind the apex. They are cut off from the proximal end of inner cortical and outer medullary cells (Fig. 73) approximately nine axial cells behind the apex. Rhizines are abundant in the inner cortex and outer medulla (Fig. 71), and later grow between medullary cells (Fig. 67). They

are unicellular, unpigmented, refractive, elongate (measured to at least 970 μm and most probably are longer), have a narrow diameter (3-4 μm), very thick walls (1.1-1.5 μm), and do not form secondary pit-connections with adjacent cells.

c. Reproductive Anatomy

Tetrasporangial stichidia are produced on upright axes along with the vegetative branches (Fig. 76). Unbranched or once branched, 1.5 mm long and 1 mm wide, stichidia are usually determinate and arranged distichously, subopposite to alternate, cylindrical to compressed, ovate to lanceolate with a blunt-rounded to blunt-tapered apex (Fig. 75). Occasionally the stichidial apex continues to grow beyond a fertile area and produces another tetrasporangial area a short distance beyond the original patch (Fig. 74), however, usually the apex does not continue vegetative growth but produces one to three short stichidial branchlets just proximal to the original fertile area. Tetrasporangial stichidia may be produced on narrow or wide plants over one cm tall and may be present on any branch order, but usually are not present on the main axis if there is more than one branch order.

Tetrasporangia are scattered randomly, i.e. not arranged in rows or V pattern, over the stichidial surface. There are generally more mature tetrasporangia in the basal part of the stichidium as these differentiate first, but, after release of the first-formed sporangia, secondary production of tetrasporangia results in a mixture of sporangia of different ages (Figs. 79, 81). Tetrasporangia occur around the entire diameter of the axis, and a sterile stichidial margin is lacking (Fig. 75).

Six to seven axial cells proximal to the apex (Fig. 77) tetrasporangia are initiated from inner cortical cells (Fig. 79). They are terminal on one of the two branches borne by a cortical cell. The sporangial pit-connection is initially basal (Fig. 79), but becomes lateral (Fig. 83) by the basal expansion of the sporangium below the pit-connection. The first division of the tetrasporangium is transverse (Fig. 81), and the second division produces four cruciately arranged tetraspores (Fig. 78), 46-89 μm long and 25-46 μm wide. Spores are released through small holes, smaller than spore widths, (diameter 5.1-10.0 μm) in the sporangial wall (Fig. 80). Either spores must be squeezed during release, or holes are stretched. The space remaining after spore release contains a substance that stains weakly with aniline blue and appears to have been partially drawn or squeezed out of the cavity (Fig. 83). Based on similar observations of other workers studying tetrasporogenesis of red algae (Peyrière, 1970; Pueschel, 1982), this substance is probably mucilage or polysaccharide from sporangium wall breakdown or a spore secretion.

Gelidium vagum is monoecious, with male and female gametes consistently formed in adjacent sections of apical parts of gametophyte branches (Fig. 85); no fertile gametophytes have been seen that produce only one sex of gametes. Gametangia are borne on short, determinate stichidia of upright axes (Figs. 82, 84), but occasionally stichidia bear two more stichidial branchlets. Stichidial apices occasionally may continue growth after a cystocarp has been produced and give rise to another patch of spermatangia and carpogonia 1.5-3 mm distal to the first gametangia. Gametangial stichidia are distichous and irregularly alternate to subopposite on second or third order branches (except on very small, 1-2 cm tall

plants where they are on the main axis). Spermatangia are located just proximal to the area that produces carpogonia (9-10 axial cells or 70-250 μm behind the apex). At low magnification fertile male areas can be recognized as areas with a somewhat raised and thickened cuticle that corresponds to the layer of unpigmented spermatangia (Fig. 85).

Carpogonia are produced three to six axial cells (50-220 μm) proximal to the apex, and the region of carpogonial production extends proximally along the length of 4-5 axial cells (100-300 μm). Carpogonia are elongate (10-25 μm long, and 3-6 μm wide at the widest, basal part), curve forward and outward and taper gradually to a trichogyne that extends directly to the thallus surface (Fig. 90). The carpogonium is intercalary, the second to basal cell of a third order filament, and has two pit-connections, one to the next distal cell and one to the next proximal cell of the filament (Fig. 90). All other cells of the filament appear unmodified. A double row of carpogonia (from both lateral sides of the axial row) are directed toward the upper branch surface and another double row are directed to the lower branch surface because third order filaments extend both above and below the second order plate (Fig. 89). In the vegetative branch, cells in the same position as the carpogonium have one proximal pit-connection (to the basal cell of the file) and two distal pit-connections (to a distal cell in the same file and to a higher order file), but in the gametangial branch the carpogonium replaces one file and its subsequent branches, creating a gap in the branch surface. The alignment of gaps from adjacent carpogonia forms an axial furrow through which trichogynes protrude.

Associated with carpogonia and young carposporophytes are short, curved chains of two to four isodiametric cells, referred to as "nutritive filaments" (Fig. 88) (Hommersand and Fredericq, 1988). These are cut off from basal cells of third order filaments and curve toward the second order plate. A lobed, multinucleate fusion cell develops after fertilization, from the fusion of the carpogonium and cortical and subcortical cells (Fig. 92). Fusion cell lobes cut off gonimoblast initials that develop into gonimoblast filaments composed of small, elongate cells that do not stain darkly with aniline blue. Gonimoblast cells fuse with apical cells of nutritive filaments. The gonimoblast branches extensively around and between cells of the second order plate, producing single, obpyriform carposporangia, 35-75 μm long and 15-25 μm wide in one of two locules formed between the second order plate and cortex (Fig. 93). A large quantity of polysaccharide or mucilage, staining faintly with aniline blue, surrounds the carpospores, much of it remaining in the locules after carpospore release. A more detailed description of carpogonium and carposporophyte development is given in Chapter 5.

As the cystocarp swells, third order filaments between the cortex and second order plate (Fig. 91) stretch and are broken, in contrast to *Gelidium purpurascens* cystocarps where filaments stretch but remain intact. Mature cystocarps are wider, 300-550 μm in diameter, than the vegetative part of the bearing branch, bilocular, and locules are separated by the plate of second order filaments (Fig. 93). The carposporophyte is restricted to the area around the second order plate. The cystocarp is domed at the ostiole, but not apiculate, and has one ostiole per locule (Fig. 93).

Spermatangia are cut off from spermatangial mother cells over most of the stichidial surface for 150-500 μm immediately proximal (9-10 axial cells or 70-250 μm proximal to the apex) (Fig. 85) to the carpogonial area. Stichidia may include some small patches of vegetative cortical cells. At low magnification, fertile male areas can be recognized by their raised and thickened wall, corresponding to the layer of unpigmented spermatangia and mucilage (Fig. 85). Spermatangial mother cells are narrow and elongate (Fig. 86), and each produces a single spermatangium by a periclinal (transverse) division. A clear, rounded body corresponding to a spermatangial vacuole can be seen basally in some spermatangia. Each spermatangium releases a single spermatium (Fig. 87) 1.5-2.5 μm in diameter. Spermatia are hemispherical to blunt-conical at release, later becoming spherical. From the first-formed (most proximal) spermatangial mother cells to the youngest (most distal) along the length of male area of the stichidium can be seen a progression and intermixing of spermatangial developmental stages, suggesting percurrent production of spermatangia.

d. Discussion

The bilocular cystocarp of this species indicates placement in *Gelidium*. Supporting this generic placement, apical and lateral initials are morphologically different and neither is in a cortical indentation. The monoecious nature of gametophytes of this species does not correspond to any *Gelidium* species previously reported from British Columbia. *Gelidium purpurascens* is morphologically distinct and dioecious. I have seen separate male and female gametophytes of *G. purpurascens* (male: UC 305373; female: UC 93572, UC 276633, UC 296689, UC 305364, UC 305371),

and *G. contortum* Loomis, *G. distichum* Loomis and *G. irregulare* Loomis, all considered synonyms of *G. purpurascens*, are known to be dioecious (Silva, 1978). *Gelidium coulteri* is dioecious and morphologically distinct (Macler and West, 1987), and *Pterocladia caloglossoides* also is morphologically distinct.

Monoecy has not been reported previously in *Gelidium*, but has been observed in *Acanthopeltis japonica* (Gelidiaceae) (Kaneko, 1968). Monoecy is a consistent feature of *G. vagum* from British Columbia and gametes are abundantly functional (judging from the large number of fertile cystocarps), eliminating the possibility of the observed condition being a genetic aberration. There are, however, a large number of *Gelidium* species for which males are unknown. Two such species, *G. vagum* from the northwestern Pacific (Japan and China) and *G. johnstonii* from the Gulf of California, show a striking morphological resemblance to the British Columbian monoecious *Gelidium*.

Illustrations of *Gelidium vagum* from China (Bangmei *et al.*, 1983; Santelices, unpubl. mscr.) and Japan (Okamura, 1934; Segawa, 1959) are morphologically similar to the monoecious *Gelidium* from British Columbia. In particular, the original description and figures of *G. vagum* (Okamura, 1934 pl. 25) described a morphology completely within the range of form displayed by the British Columbian monoecious species. British Columbian plants also agree with Santelices' (unpubl. mscr.) description of Chinese *G. vagum*. To date, Okamura has provided the only illustrations of a cystocarpic plant and a cystocarp, but the diagnosis lacks illustrations or mention of internal cystocarp anatomy.

Because the type of *Gelidium vagum*, in the Makino Herbarium of the Tokyo Metropolitan University (MAK; Akatsuka, pers. comm.), was unavailable for loan, other specimens of Japanese *G. vagum*, borrowed from the National Science Museum, Tokyo [TNS 25817, TNS 25823 (Fig. 94), TNS 25824, TNS 25825, TNS 25847] and a single sheet with two to four Japanese plants in UBC (A56807), were examined. Two plants are cystocarpic [TNS 25824 (Fig. 95), TNS 25825], and when sectioned were found to be monoecious (Fig. 96). Spermatangia in these plants are like those in the British Columbian monoecious species, occurring just proximal to the female gametangial area, and later at, and extending below, the cystocarp base. Cystocarp anatomy also is similar in British Columbian and Japanese plants (Fig. 96). Occasionally, however, cystocarps of Japanese plants have an intact third order filament between the second order plate and cortex (arrowhead in Fig. 97), whereas equivalent filaments in British Columbian material are broken during cystocarp expansion.

As in the British Columbian material, tetrasporangial stichidia of *G. vagum* lack a sterile margin, and have tetrasporangia scattered irregularly over the stichidial surface (Fig. 98). Tetrasporangial stichidial anatomy is similar in both entities, although in Japanese plants, tetrasporangia are embedded somewhat more deeply in the thallus with one or two cortical layers lying outside the outer end of sporangia (Figs. 99, 100).

In light of the similarity of other characters, i.e. the morphology of thalli with regard to branching pattern, branch size, shape and degree of constriction, presence of male and female gametangia not only on the same thallus (monoecy)

but in the same arrangement, the absence of a sterile margin in tetrasporangial stichidia and the arrangement of tetrasporangia in stichidia, the difference observed in breakage of sterile filaments linking cells of the second order plate and cortex, and in the degree of tetrasporangia embedding are considered minor. Thus, this monoecious British Columbian *Gelidium* is considered to be conspecific with *G. vagum*.

Spermatangia also were unknown for *Gelidium johnstonii* Setchell et Gardner (1924: 742),¹ when it was described from the Gulf of California, and illustrations (Setchell and Gardner, 1924 pls. 46a, 72, 73) showed a strong morphological resemblance between this species and *G. vagum*. Akatsuka (1986b) suggested that *G. vagum* was related closely to *G. johnstonii*. Examination of the holotype (Fig. 101), isotypes and paratype, as well as specimens identified as *G. johnstonii* collected by Dawson (AHFH 2211, AHFH 4150, AHFH 4156, AHFH 4179, AHFH 4192, AHFH 4193, AHFH 4194, AHFH 50267, AHFH 50268, AHFH 50299, LAM 52684 in AHFH, LAM 52894 in AHFH), revealed that small, to 5 cm tall, plants resemble *G. vagum* morphologically, but that larger plants are more robust and coarse, lacking the delicate appearance of *G. vagum*. *Gelidium johnstonii* and *G. vagum* differ anatomically in several vegetative and reproductive features listed below and summarized in (Table 6). Contrary to Setchell and Gardner (1924) the medulla of the holotype of *G. johnstonii* is not sparse, however the outer cortex is three to four cells as they had reported (Figs. 102,

¹Holotype: (Johnston 27) CAS 1343 in UC!

Isotypes: (Johnston 27) CAS 484385 in UC!, CAS 484386 in UC!, CAS 484388 in UC!

Paratype: (Johnston 13) CAS 484390 in UC!

Type locality: Bahia San Francisquito, Gulf of California, Baja California del Norte, Mexico

103). In contrast, the outer cortical cells of *G. vagum* are unaligned.

Tetrasporangial stichidia of *Gelidium johnstonii* are elongate, to 3 mm, spatulate (Fig. 106), flattened, and have a blunt apex (Fig. 104) and a sterile margin of variable width (Fig. 104) unlike the shorter, ovate, terete to compressed stichidia with more pointed apices of *G. vagum*. The apical initial of *G. johnstonii* is domed and protrudes slightly beyond adjacent cortical cells (Fig. 105), but in tetrasporangial and gametangial stichidia the apical cell is level with the adjacent cortex or recessed slightly between adjacent cortical lobes (Fig. 109). In *G. vagum*, the apical initial of both vegetative branches and fertile stichidia protrudes beyond adjacent cortical cells (Figs. 68, 75). In *G. johnstonii* a furrow develops immediately behind the apical cell in gametangial and tetrasporangial stichidia (Fig. 105, 109) which is not filled in by cells of higher order filaments for 250-350 μm behind the apex. An apical furrow is absent in *G. vagum* tetrasporangial stichidia (Fig. 75), but is present in gametangial stichidia, as a result of cortical cells being replaced by carpogonia (Fig. 89, development described earlier). The furrow in *G. vagum* gametangial stichidia is not analogous developmentally to the furrow in *G. johnstonii* tetrasporangial stichidia.

The mature cystocarp of *Gelidium johnstonii* (Fig. 107) has stretched but intact third order filaments (Fig. 108), as in *G. purpurascens*, and differs from *G. vagum* where third order filaments are broken. Significantly though, *G. johnstonii* is monoecious, male and female gametes appearing in the same position as in *G. vagum* (Fig. 111).

Despite the morphological similarity of smaller thalli of *Gelidium vagum* and *G. johnstonii*, and that both species are unique in being moneocious, these taxa are regarded as separate species because of several consistent and significant character differences (Table 6). Since they are both moneocious, a condition that is otherwise unknown in Gelidiales, and because of the uniformity of spermatangial position with respect to carpogonia, it is reasonable to propose that *G. johnstonii* and *G. vagum* are closely related sister species.

A similar pattern of warm-temperate to sub-tropical, East Pacific—West Pacific disjunct distributions has been observed for *Pachydictyon coriaceum* (Holmes) Okamura, *Tinocladia crassa* (Suringar) Kylin, *Endarachne binghamiae* J. Agardh, *Prionitis cornea* (Okamura) Dawson, *Ishige sinicola* (Setchell et Gardner) Chihara and *Lomentaria catenata* Harvey, and for the species pairs *Eisenia arborea* Areschoug and *E. bicyclis* (Kjellmann) Setchell, and *Carpopeltis divaricata* Okamura and *Binghamia californica* J. G. Agardh (Hommersand, 1972). In addition, numerous other species pairs have been suggested on the basis of preliminary observations (Hommersand, 1972), and *Gelidium johnstonii* and *G. tenue* Okamura (1934: 56) are found as a pair of taxa. The description and illustrations of *G. tenue* (Okamura, 1934, pls. 23, 31 fig. 8-10) are not strikingly reminiscent of *G. vagum*, and a similarity between *G. tenue* and *G. vagum* has not been noted in discussions of Japanese or Chinese species of *Gelidium* (Akatsuka, 1986b; Santelices, unpubl. mscr.). No evidence has been presented that supports a sister taxa relationship between *G. tenue* and *G. vagum*.

The combination of the 1) narrow range of distribution in the Pacific Northwest,

restricted to British Columbia of *Gelidium vagum* (see below), and 2) its great abundance and vigour within its distribution, suggests that this species may have been a recent introduction rather than a relict of a population of a once wider distributional range in the northwest Pacific. Many oyster farms are present in the Hornby Island-Denman Island area. As such, the importation of Japanese oyster spat is a likely vehicle for the introduction of *G. vagum* into British Columbia. Scagel (pers. comm.) has suggested that another species, *Lomentaria hakodatensis*, of the British Columbian algal flora may have also been introduced accidentally, probably in oyster spat. In view of the rapid colonization of Hornby Island and Denman Island, future range expansion of *G. vagum* in British Columbia might be expected. To date no other west coast of North America populations are known, although a single sterile specimen of *G. vagum* (UBC A64965) (Fig. 110) appeared in the drift at Ladysmith, farther south in the Strait of Georgia.

e. Seasonality and Distribution

Gelidium vagum is perennial in British Columbia. Fertile tetrasporophytes and cystocarpic plants have been collected in the fall (August, September, October), whereas plants collected in April were infertile. Japanese specimens (TNS; UBC) were tetrasporangial in May and July and gametangial in July.

In British Columbia, *Gelidium vagum* is restricted to Hornby Island and Denman Island (Strait of Georgia).

In addition to the Strait of Georgia in the area of Hornby Island and Denman Island in British Columbia, *G. vagum* is distributed in the Huanghai Sea (Bangmei *et al.*, 1983), and the Yellow Sea of China (Akatsuka, 1987; Santelices, unpubl. mscr.), the Korea Strait and Sea of Japan, (Tokida, 1954; Akatsuka, 1987; Kajimura, 1987), and the Pacific coasts of Honshu and southern Hokkaido (Okamura, 1934; Akatsuka, 1987).

4. *Pterocladia caloglossoides* (Howe) Dawson

1953: 76

Basionym: *Gelidium caloglossoides* Howe 1914: 96, fig. 7, pls. 34, 35

Synonym: *Pterocladia parva* Dawson 1953: 77 fig. 2, pl. 6, n.v.

Holotype: Coker 59 (NY); lost on loan (Thiers, pers. comm. 1987), if no other specimens are found, a neotype should be made.

Type locality: Island of San Lorenzo, Peru

a. *Habitat and Habit*

Pterocladia caloglossoides grows in the shallow subtidal, 1-7 m depths, in moderately exposed to protected locations. It is epilithic on tops and sides of boulders, on platforms or on walls, epiphytic on crustose corallines and also occurs on calcareous shells. Plants are soft, but not lax, and are dark red, drying to blackish red. They form a dense, low turf of upright axes that arise from an extensive system of prostrate axes (Fig. 112). Many uprights develop along the length of a single prostrate axis. Although the most vigorous plants

may reach 2 cm tall, the turf usually is not more than 1 cm tall.

Branching in *Pterocladia caloglossoides* ranges from very sparse to abundant; the number of branch orders is low; uprights may be unbranched or may bear a maximum of two branch orders, though second order branches were uncommon in the study area. Branches are distichous, irregularly subopposite, and the branch angle is almost 90° (Fig. 113). Axes are compressed to flattened at branch bases, especially where branches are regenerating from cut ends. Upright axes vary greatly in width, and a single branch may range from 240-690 μm . Axes taper gradually at their bases and abruptly at their apices to an acute tip.

Prostrate and upright axes are similar, except that prostrate axes have attachment pads at frequent intervals whereas pads are lacking on upright axes. Occasionally an upright axis bends down, developing attachment pads and becoming prostrate. Branching of prostrate axes is subopposite distichous, but when producing uprights, one to five axes may arise from a single node. Uprights are perpendicular to prostrate branches and occur opposite an attachment pad (Figs. 114, 116). Prostrate axes are less variable in width (230-260 μm diameter) than upright axes and are approximately the same width as narrower uprights. Prostrate axes do not taper at branch points, but have acute apices.

Attachment of prostrate axes to the substratum is by numerous attachment pads formed medially on the ventral surface of creeping axes opposite the upright branches (Fig. 116) and formed on the lower surface of an upright branch that

is becoming prostrate. Cortical cells elongate, or produce elongate, unpigmented, rhizoid-like lobes that adhere to form attachment pads (Figs. 120, 121). Pads are to 1.0 mm long and are 0.2-0.5 mm wide and flare out distally, as the distal end of cells incorporated in the pad are swollen and bulbous.

b. Vegetative Anatomy

Thallus construction is uniaxial with a conspicuous, domed apical cell and two periaxial cells derived from each subapical cell (Fig. 115). Second order filaments grow laterally outward at a 45-60° angle to the axial filament. These are evident just behind apices where cells of second order filaments are in diagonal rows from the axial filament (Fig. 115).

Transverse sections of *Pterocladia caloglossoides* reveal a cortex of only 1-2 cell layers (Fig. 117) and a medulla. The outer wall of superficial cortical cells is as wide as the protoplast diameter. Cortical cells in upright axes are pigmented, oval or short cylindrical, 7-10 μm long and 5-10 μm wide, and in surface view are equidistant, but unordered and irregularly arranged (Fig. 119). Cortical cells of creeping axes are slightly larger, but are otherwise like cortical cells of upright axes. Outer cortical cells lack secondary pit-connections, whereas the inner cortical cells (in specimens where two rows are present) have secondary pit-connections.

The medulla is 3-5 cells thick and in upright axes composed of unpigmented, cylindrical cells, 12-27 μm long and 7-13 μm wide, that have abundant

secondary pit-connections (Figs. 117, 118). Medullary cells of prostrate axes are slightly larger. Cells of the axial filament are conspicuous in longitudinal section (Fig. 118). Rhizines, present in the medulla (Fig. 117), are very abundant in upright axes but are more sparse in creeping axes. They are initiated from the inner proximal corner of medullary or inner cortical cells, at a position 6-7 axial cells behind the apex.

c. Reproductive Anatomy

Tetrasporophytes are the only reproductive thalli collected from the study area. Gametophytes are unknown in British Columbia, northern Washington and southeast Alaska.

Tetrasporangia form only on main axes and short lateral branches of upright axes (Fig. 113), occurring in sori but are not in stichidia; the branch apex grows vegetatively beyond the sorus. Short, fertile, first order branches narrow rapidly at the base, and taper abruptly to an acute apex, similar in shape of similarly positioned vegetative branchlets. One of the most striking features of *Pterocladia caloglossoides* is the characteristic "V" arrangement of tetrasporangia in the sorus (Fig. 122). The more proximal V's are oldest. Sporangia occur in pairs of lines radiating laterally at 45° to the axial filament. The margin of the flattened branch is sterile, with tetrasporangia borne on both faces of branches which are recognizable approximately five axial cells behind the apex. They are cut off from medullary cells adjacent to the second order plate (Fig. 123) and have a lateral pit connection (Fig. 124) to their bearing cell. Tetrasporangia are

crucially divided (Fig. 125), sometimes irregularly, and 20-45 μm long and 15-40 μm wide. Only rarely do young sporangia form between cavities of released sporangia, indicating that percurrent production of tetrasporangia is uncommon in *P. caloglossoides*.

d. Discussion

Pterocladia caloglossoides originally was described by Howe (1914) as *Gelidium caloglossoides* from the Island of San Lorenzo, Peru. It was found on shells dredged from 2.5 fathoms (5 m depth) by Dr. Robert E. Coker. The holotype (Coker 59 in NY) was lost while on loan (Thiers, pers. comm. 1987) and it is unknown if isotypes exist. I have written to AHFH, BM, FH, MICH, NY and US, but their curators have not located isotypes of *G. caloglossoides*. Coker 59 was the only material mentioned by Howe in the diagnosis. Questions may be raised of the connection between the taxon Howe described as *G. caloglossoides* from Peru and Dawson's identification of material from Pacific Mexico as *P. caloglossoides* that was used in comparison with British Columbian *P. caloglossoides*. In Howe's original diagnosis, *G. caloglossoides* was characterized by its creeping habit, small size, radiate clusters of 2-5 upright branches opposite attachment pegs, flattened axes, surface cells in distinct oblique rows at branch apices, narrow medulla, and tetrasporangia in "distinct oblique lines". Gametophytic plants either were not found or described. Howe noted the similarity of *G. caloglossoides* to *G. pusillum* but believed that *G. caloglossoides* was sufficiently distinct on a number of criteria to warrant recognition as a separate species.

Hollenberg (1942) reported *Gelidium caloglossoides* from southern California and Monterey, central California., which was included by Smith (1944) in the marine flora of the Monterey Peninsula. Diagnostic features used to recognize this taxon were its prostrate habit, small size, flattening of the branches, branch pattern and arrangement of tetrasporangia in V's. Smith (1944) also noted that in the Monterey specimens the upright axes have few orders of branching and that branches are almost perpendicular to main axes. Dawson (1953) transferred *G. caloglossoides* to *Pterocladia* based on unilocular cystocarps found in material from Pacific Baja California del Norte, observing that the tetrasporangial plants from Baja California were the same as the material described from Peru by Howe (1914).

I have compared British Columbian *Pterocladia caloglossoides* with Dawson's material from Pacific Mexico (AHFH Dawson 8593, liquid-preserved and specimens on microscope slides 1176, 1177 from Guadalupe Island, off Baja California, and AHFH Dawson 8733 liquid-preserved and microscope slides 1325, 1326, 1327 from Barra de Navidad, Jalisco on the mainland of Mexico). British Columbian *P. caloglossoides* resembles to both these collections, although it is most similar to the Guadalupe Is. plants. Specimens from Guadalupe Is. were a maximum of 1 cm tall, with unbranched uprights, the tallest of which were undamaged by grazing or abrasion. Liquid-preserved Guadalupe Is. specimens, those on slide 1176 and three of four plants on slide 1177 are tetrasporophytes, with tetrasporangia clearly in V's (Fig. 126). On the creeping axes are numerous attachment pads opposite from which two to four upright branches arise (Fig. 114). These Pacific Mexico plants fit easily within the description of *P.*

caloglossoides given and illustrated by Howe (1914). Baja California plants are less well developed than those from Peru, but British Columbian plants from Orlebar Point, Gabriola Island, where frequent collections were made throughout the year, showed much variation in the development of upright axes from their almost complete absence to unbranched axes, and to well branched axes. British Columbian *P. caloglossoides* also corresponds well with all other illustrations and descriptions given for central California, and Pacific Mexico and Gulf of California *P. caloglossoides* (Smith, 1944; Stewart, 1976; Stewart and Norris, 1981), and thus is determined to occur in the study area.

One plant of Dawson's slide 1177 from Guadalupe Is. (Fig. 127) is cystocarpic. Its morphology matches that of tetrasporophytes, and it is reasonable to assume that the specimen is representative of *Pterocladia caloglossoides*. From what can be seen from a whole mount of a cystocarpic plant (giving a "top" view of a cystocarp), an ostiole appeared on only one face of the cystocarp (Fig. 129), indicating a single locule. Sections of a cystocarp from liquid-preserved material (AHFH Dawson 3733) show a single locule (Fig. 128). Thus the species as known from Pacific Mexico does indeed belong in *Pterocladia*, and Dawson (1953) was justified in transferring *Gelidium caloglossoides* to *Pterocladia*, though he admits that absolute confirmation of placement in *Pterocladia* could only be obtained by observing cystocarps in type locality Peruvian material. It is interesting that Dawson later used the combination *G. caloglossoides* when describing specimens from the Peruvian flora (Dawson *et al.*, 1964).

Added evidence for the generic placement of this taxon in *Pterocladia* comes from

apical and lateral initial morphology observed in British Columbian material. Both types of initials are domed hemispherical and protruding, and neither occur in cortical depressions. This finding is in agreement with Rodriguez and Santelices' (unpubl. mscr.) proposal that apical and lateral initials are similar in shape in *Pterocladia* and dissimilar in *Gelidium*, but it contradicts their statement that either the apical or lateral initials are depressed in cortical indentations in *Pterocladia*. The "indentation of initials" character while apparently reliable for other taxa of *Pterocladia* was not corroborated in British Columbia *P. caloglossoides*.

Pterocladia caloglossoides as identified by E. Y. Dawson from Barra de Navidad, Jalisco, Mexico (AHFH Dawson 8733) are heavily damaged from abrasion or grazing, with truncated uprights that only reach a maximum of 0.5 mm tall. Some of the truncated axes have regenerated apical cells, but little regrowth occurred before the plants were collected and preserved. Two plants (slide 1327, AHFH) have many attachment pads and several uprights opposite the pads. Sections of vegetative plants (Fig. 132; slide 1326, AHFH) show a cortex and medulla that is well developed compared to British Columbian *P. caloglossoides*. Dawson's material does correspond to the illustrations and description given by Howe for *Gelidium caloglossoides*, particularly in the distinctive features of branching of the prostrate axis, attachment pad position and arrangement of tetrasporangia in V's. Thus I agree with Dawson (1953) that the Mexican material with a single cystocarp locule is the same as *G. caloglossoides* (Howe, 1914) and it belongs in *Pterocladia*.

Dawson (1953) noted that "antheridia" were unknown in *Pterocladia caloglossoides*, however, specimens of his (slide 1325, AHFH) are a whole mount of one female and two male thalli. The fertile apices of these gametophytes are blunt rounded (Fig. 129), whereas vegetative apices are more acute and tapering. Spermatangia are in clusters of two to four in sori behind apices (Fig. 130). Each cluster replaces a vegetative cortical cell (Fig. 131), and spermatangia are cut off transversely and singly from spermatangial mother cells (Fig. 133).

When Dawson made the new combination *Pterocladia caloglossoides*, he also described a new species, *P. parva* Dawson (1953: 77)¹ based on only female gametophyte material. Dawson's illustration (1953, pl. 6 fig. 2) of *P. parva* is strongly resembles *P. caloglossoides*, an observation supported by Dawson's comment that the illustration of *P. caloglossoides* in Smith (1944, pl. 44 figs. 3, 4) is more similar to *P. parva* than to *P. caloglossoides*. *Pterocladia parva* later was considered a synonym of *P. caloglossoides* by Stewart and Norris (1981).

The similarity of *Gelidium pusillum* to *Pterocladia caloglossoides* was noted by Stewart (1976) and Stewart and Norris (1981), because both are small and turf-like. In California, however, *G. pusillum* has bilocular cystocarps, characteristic of the genus *Gelidium*. The name *G. pusillum* was used with some reservation (Stewart, 1976) for material from California. Other species names, *G. latifolium*, *G. sinicola*, *P. parva* and *P. caloglossoides* have been applied to this California material, although more definite determinations necessarily await a thorough comparison with type material of *G. pusillum*.

¹Holotype: Dawson 425 on sheet 4181, plus slides 1178-1181 in AHFH
Type locality: San Felipe, Baja Californiadel Norte, Gulf of California

e. Seasonality and Distribution

Pterocladia caloglossoides is perennial, persisting for most of the year as prostrate axes with few uprights that become more abundant and form a turf in the summer months, reaching their maximum development in August and September. Tetrasporangial sori are most abundant at this time. A collection was made in February 1987 at Georgina Point, Mayne Island (Fig. 3), however, in which tetrasporangial sori were present but sparse.

Pterocladia caloglossoides is relatively common and widespread in the study area, but it likely has been overlooked and not reported from many sites due to its small size and creeping habit. This species has been collected from Orcas and San Juan Islands in northern Puget Sound (Fig. 3), Mayne and Gabriola Islands in the Gulf of Georgia (Fig. 3) and Hornby and Denman Islands in the Strait of Georgia (Fig. 4), Sooke (Juan de Fuca Strait), Barkley Sound (Fig. 2) and Nootka Island, on the west coast of Vancouver Island, and the Queen Charlotte Islands (Fig. 1).

This study extends the northern limit of distribution of *Pterocladia caloglossoides* from northern Washington (Norris and Hollenberg, 1969) to British Columbia (e.g. UBC A12295, UBC A19667, UBC A28937, UBC A37605, UBC A64648, for others see Appendix 1) and southeast Alaska (UBC A69423, UBC A69424, UBC A69425, UBC A69426, UBC A69427, UBC A69428, UBC A69529, UBC A69430, UBC A69431). *Pterocladia caloglossoides* also is reported from Hawaii (Santelices, 1977), the Great Barrier Reef, Australia (Cribb, 1983), the Indian

Ocean south of Sumatra (Weber van Bosse, 1921), and Natal, South Africa (Norris, 1987).

D. PREVIOUSLY REPORTED TAXA EXCLUDED FROM THE REVISED FLORA

Gelidium crinale, *G. pusillum*, *G. robustum* and *G. sinicola* have been reported in the local flora (Widdowson, 1974; Scagel *et al.*, 1986), but these are based on taxonomic misidentifications as none of these taxa actually occur in the study area. With the exception of *G. robustum*, these taxa are generally poorly understood and their species limits poorly defined.

In the first two decades of this century, *Gelidium amansii* (type locality "in mari Indico ad ins. Franciae, Madagascar, Indiae orientalis", Kützinger, 1849) was reported from southern British Columbia and northern Washington (Setchell and Gardner, 1903; Collins, 1913; Kylin, 1925; Connell, 1928), and reported plants were likened to P. B.-A., No. 585 (distributed as *G. amansii*, Collins *et al.*, 1903, but in fact are now recognized to be *G. robustum* collected in California) by Setchell and Gardner (1903). Collins (1913) noted that British Columbian plants based on collections by Macoun from Ucluelet, Departure Bay and Victoria and by Tilden from Port Renfrew (Minnesota Seaside Station) he referred to *G. amansii* were much smaller than Californian specimens. Without exact collection data confirmation of Collins' determination cannot be made but UBC and CANA have Macoun and Tilden collections from these locations (Macoun: UBC A1952, CANA 3843, CANA 4346, CANA 4349 (Fig. 135); Tilden: UBC A5195). All of

these specimens were determined to be *G. purpurascens*. Kylin (1925) included *G. amansii* in the Friday Harbour flora but had not seen any specimens. These early records of *G. amansii* have been treated by later authors as misidentifications (Scagel, 1957).

There is a single report of *Gelidium pulchrum* from the area of Nanaimo (Stephenson and Stephenson, 1961b). Vouchers of this record do not exist and the Nanaimo area has been recollected without finding any specimens that are this taxon. This record has been included as misidentified *G. purpurascens* by Scagel *et al.* (1986) in the most recent floristic treatment of British Columbia, northern Washington and southeast Alaska.

In the following discussion, I treat those taxa more recently reported for the local flora but that are, in fact, absent, based mostly on taxonomic misidentifications.

1. *Gelidium robustum* (Gardner) Hollenberg *et* Abbott

1965: 1179

Basionym: *Gelidium cartilagineum* var. *robustum* Gardner 1927a: 280, pl. 54

Holotype: UC 294572!

Type locality: near Ensenada, Baja California del Norte, Pacific Mexico

Gelidium robustum is a well defined species ranging from central Baja California, Mexico (Stewart, 1976) to central California (Silva, pers. comm.). First was

described by Gardner (1927a) as a variety of *G. cartilagineum* (L.) Gaillon, it was later elevated to species rank by Hollenberg and Abbott (1965). Based on an examination of the holotype from near Ensenada, Baja California del Norte (UC 294572, Fig. 138) and additional representative material (UC 395419 from San Pedro, California collected by Gardner; UC 647822, from White Pt., California collected by Dawson; and UC 756464, UC 756469, UC 756470, UC 756503, and UC 940173 from various localities in Baja California, Mexico collected by Dawson; and UC 1451987 from Portuguese Bend, California collected by Loomis), it was concluded that none of the British Columbian plants (with the possibility of one exception) correspond to this taxon.

There is an interesting specimen in UBC (UBC A7861) of a plant that is tall, robust, tetrasporangial and clearly is *Gelidium robustum* (Fig. 136). It poses somewhat of a mystery. The annotation label states the collection locality as "Shoal B. Victoria" from 1917 by an unknown collector. There is no "Shoal B." near Victoria, British Columbia (Canadian Coast Pilot) although it is unclear whether the reference is to Shoal "B", or whether B is an abbreviation for bay, bight, beach etc.. However, there are no such locales near Victoria, B.C., but there are some in the San Juan Islands a few km to the east of Victoria (Canadian Coast Pilot). A considerable amount of seaweed collecting was done in southern Vancouver Island as early as 1917, and it is unlikely that if *G. robustum* existed at Victoria in 1917, such a large, conspicuous plant would have been collected only once and never at any time between central California and British Columbia. Admittedly, a small population could have been in Victoria (perhaps introduced from California in the early 1900's), that has subsequently

disappeared. It is also possible that collection data were confused between samples from a variety of locations along the coast. As it stands, even if *G. robustum* had been present in British Columbia, it does not exist here now, and *G. robustum* must be excluded from the flora.

In the study area, several specimens of *Gelidium purpurascens* were misidentified as *G. robustum*. Differences between these taxa already have been discussed (Table 4). Several of the literature records alluding to the presence of *G. robustum* in the study area lacked voucher specimens or illustrations, and those records could not be investigated. None of the reports for which there are vouchers identified as "*G. robustum*" were in fact *G. robustum*, but are misidentifications. Scagel (1957) cited five specimens from Departure Bay and Victoria on Vancouver Island (as *G. cartilagineum* var. *robustum*) (CANA 3473=CAN 209, CANA 3740=CAN 74, CANA 3843=CAN 353, CANA 4346=CAN 208, CANA 4349=CAN 310), and all were re-examined and found to be *G. purpurascens*, and Scagel's (1967) illustration is of *G. purpurascens*. South's (1968) records of "*G. robustum*" from Gabriola Island were of a variety of taxa: specimens from Orlebar Point (UBC A28937, UBC A29575) are *Pterocladia caloglossoides* and *G. coulteri* respectively, specimens from Lock Bay (UBC A36199) and False Narrows (UBC A36198) are *G. coulteri* and specimens from Twin Beaches (UBC A28645, UBC A28646) are *G. coulteri* and *G. purpurascens* respectively. All records of "*G. robustum*" from Barkley Sound (Scagel, 1973) that could be located were found to be *G. purpurascens*.

2. *Gelidium crinale* (Turner) Lamouroux

1825: 191

Basionym: *Fucus crinalis* Turner 1819: 4Synonyms: *Acrocarpus crinalis* (Turner) Kützinger 1868: 11, pl. 33 figs. a-c, n.v.*Acrocarpus spinescens* Kützinger 1868: 12, pl. 33 figs. d-e, n.v.*Acrocarpus corymbosus* Kützinger 1868: 13, pl. 36 figs. a-c, n.v.

Lectotype: Specimen annotated by Setchell as "This is Turner's idea of *G. crinale* and may be taken as type. f. W.A.S." (from Turner Herbarium in BM) (Dixon and Irvine, 1977b)

Isotype: AHFH 55234, n.v.

Type locality: Kilmouth, England

Descriptions of *Gelidium crinale* (Gardner, 1927a; Dawson, 1944, 1953; Taylor, 1957; Santelices, 1977) demonstrate the lack of well defined diagnostic characters for this species. Santelices (unpubl. mscr.) especially has noted the confusion about the species limits and identifications of this taxon, and the resulting misapplications of the name. Collins (1913) first recorded *G. crinale* in the study area from Victoria, B.C. and cited P. B.-A. No. 195 (Collins, *et al.*, 1896, Fasc. IV) from Port Jefferson, Long Island, New York, *G. crinale* as a reference specimen, but this specimen is different from any species present in British Columbia or northern Washington. Collins' record for *G. crinale* is based on a Macoun collection from Victoria (Collins, 1913), which is probably CANA 3474 (formerly CAN 207) (Figs. 134, 137) from Beacon Hill, Victoria, collected June 1908 and labelled as *G. crinale*. Re-examination of the plants on this sheet

revealed them to be *G. coulteri*.

The inclusion of *Gelidium crinale* in the British Columbia flora has continued with the repeated citation of CANA 3474 (as CAN 207); for the record from Departure Bay, B.C. (as V 1456) (Scagel, 1957), the specimen could not be located. A specimen (UBC A66778) collected by Scagel from Departure Bay as "*G. crinale*" probably corresponds to the missing V 1456 and is stamped "on loan from the B.C. Provincial Museum". This specimen is now recognized to be *G. purpurascens*. In UBC there are numerous specimens misidentified as "*G. crinale*" that were determined to be *G. coulteri* (UBC A31363, UBC A31426, UBC A37402, UBC A53975, UBC A60254, UBC A60268, UBC A60460, UBC A64934), and one that is *Pterocladia caloglossoides* (UBC A19667). Many other specimens originally labelled as "*G. crinale*" have been annotated as other species, e.g. *G. coulteri*, *G. purpurascens*, *P. caloglossoides*, reflecting the taxonomic confusion of *G. crinale*, and the assumption of its presence in the local flora. time of the collection.

Gelidium crinale first was recorded from California by Collins (1903 in Collins *et al.*, P. B.-A., Fasc. XXIII, No. 1138), and it was the basis for a new variety, *G. crinale* var. *luxurians* Collins (1906: 111). Later authors continued to record the presence of *G. crinale* var. *luxurians* from California (Gardner, 1927a) and Baja California del Norte, Mexico (Dawson, 1953). Dawson (1944, 1953, 1966) listed both *G. crinale* (var. *crinale*) and *G. crinale* var. *luxurians* from Pacific Mexico and the Gulf of California.

Stewart (1974) considered the Californian *Gelidium crinale* var. *luxurians* (i.e. P. B.-A., Fasc. XXIII, No. 1138; and the material mentioned in Gardner, 1927a) to be *Pterocladia media* Dawson (1958: 68). The remaining specimens of "*G. crinale* var. *luxurians*" Dawson and *G. crinale* var. *crinale sensu* Dawson (1944, 1953) were considered *G. pusillum* by Stewart and Norris (1981). Thus *G. crinale* var. *crinale* and *G. crinale* var. *luxurians* do not occur in the Gulf of California or in the northeast Pacific.

3. *Gelidium pusillum* (Stackhouse) Le Jolis

1863: 139

Basionym: *Fucus pusillus* Stackhouse 1801: 6

Synonyms: *Fucus caespitosus* Stackhouse 1801, pl. 12 n.v.

Acrocarpus pusillus (Stackhouse) Kützinger 1849: 762 n.v.

Lectotype: in BM (Dixon and Irvine, 1977b)

Type locality: Sidmouth, England

There is no evidence to support the presence of *Gelidium pusillum* in the study area. Of the reports of "*G. pusillum*" from British Columbia and northern Washington (given in Scagel *et al.*, 1986), only Norris and Wynne (1968) cited voucher specimens and Garbary *et al.* (1984) did not specify which UBC herbarium specimens they used. Norris and Wynne (1968) first reported *G. pusillum* in the study area from San Juan Island, Washington, but the specimen they reported (WTU, Norris 5723) could not be located. Collections other than those of Norris and Wynne (1968) of gelidioid plants from San Juan Is. belong

to *G. purpurascens* (UBC A4402, UBC A4403; FHL 2849; private herbarium of W.R. Waaland #1583) and to *Pterocladia caloglossoides* (WTU 248018). The Scagel *et al.* (1986) report of "*G. pusillum*" from Barkley Sound, based on a specimen (UBC A61245), is now identified as *P. caloglossoides*. Other herbarium specimens from the study area labelled as "*G. pusillum*" are mis-identifications, and actually *G. coulteri* (UBC A24837, UBC A28351, FHL 3055) and *P. caloglossoides* (UBC A33645, UBC A64648).

Gelidium pusillum has a worldwide distribution and, like *G. crinale*, is poorly circumscribed (Santelices, unpubl. mscr.). Different workers have used varying species concepts that have incorporated numerous other species (Dixon and Irvine, 1977a; Stewart, 1976; Stewart and Norris, 1981), or they have recognized many varieties (Dawson, 1944, 1953; Santelices, 1977; Schnetter and Bula Meyer, 1982). The name seems to have been applied commonly to any small, turf-forming, compressed to flattened gelidiaceous plants. This taxon clearly needs revision on a global scale, beginning with a thorough study of the type material.

4. *Gelidium sinicola* Gardner

1927a: 278

Holotype: (Gardner 2615) as UC 276620!

Type locality: Point Cavallo, San Francisco Bay, California

Records of "*Gelidium sinicola*" in the study area result from confusion as to the definition of the taxon. *Gelidium sinicola* Gardner (1927a) was described as

"sparse" (i.e. not abundant) and "of limited distribution", and based only on the type locality collection from Point Cavallo, San Francisco Bay, California (Gardner 2615 as UC 276620) (Fig. 139). Even Gardner was apparently confused as to the identity of this species. His later collections from the same tidepool as the type collection (Gardner 7179, UC 494898) (Fig. 21), were noted in his field book "*G. sinicola* ? at least from the same pool as type came" (Silva, pers. comm. 1985), and on the herbarium sheet he identified the plant only as "*Gelidium*". Added later, but not in Gardner's handwriting (possibly by E. Y. Dawson, *fide* Silva, pers. comm. 1985), is "*coulteri* Harv. f."

Both the type specimen and Gardner 7179 have a morphology that could fit within the limits of *Gelidium coulteri*. *Gelidium sinicola* differs from *G. coulteri* in that it is cylindrical rather than flattened (Fig. 140), and it is narrower (Gardner, 1927a). Stewart (1976) noted that *G. coulteri* varies widely in compression of branches, and I also have seen much variation of axis width and compression in *G. coulteri* from the study area. Silva (pers. comm., 1985) has visited the type locality of *G. sinicola* several times and has made extensive collections around San Francisco Bay, and writes of seeing plants he considers to be morphologically variable *G. coulteri*, but never any cylindrical plants that could correspond to *G. sinicola*. Because the type material is infertile, there are few characters for species comparison. Based on a comparison of height, apical and basal axis diameter, branching pattern and rhizine abundance and position, in *Pterocladia media*, *G. sinicola* (holotype and Gardner's diagnosis), and Californian *G. crinale* var. *luxurians*, Stewart (1974) suggested that *G. sinicola* might be a synonym of *P. media*. Generic placement cannot be confirmed for *G. sinicola*

because no cystocarpic plants are known to exist.

Despite the problems of the actual identity of *Gelidium sinicola*, records of it in the study area are based on misidentifications of other gelidioids. Specimens in UBC labelled originally as "*G. sinicola*" belong to *G. coulteri* (UBC A1449, UBC A14403) or *Pterocladia caloglossoides* (UBC A29576, UBC A39127). Of the reports of "*G. sinicola*" in the study area (given in Scagel *et al.*, 1986), only Norris and West (1966) cite vouchers. The voucher cited by Norris and West (Norris 5087) could not be located. Thus *G. sinicola* does not appear to occur in the study area.

CHAPTER IV. LIFE HISTORY OF *GELIDIUM* IN CULTURE

A. INTRODUCTION

Until recently, the life history had not been completed successfully in culture for any species of Gelidiales. This is somewhat surprising considering the economic potential and interest in the group. Most commercial uses of Gelidiaceae have been restricted to wild harvest. It was assumed from field collections that Gelidiaceae have a *Polysiphonia*-type life history (Fig. 141) (Dixon, 1961, 1963) with a sequence of (haploid) gametophyte, (diploid) carposporophyte and (diploid) tetrasporophyte phases, where gametophyte and tetrasporophyte phases are free-living and isomorphic, and the carposporophyte develops on the female gametophyte. In order to confirm this, I attempted to culture the local *Gelidium* species. According to recent studies on the life histories of *G. coulteri* (MacIer and West, 1987) and *G. vagum* (van der Meer, pers. comm.), both species require a short time to complete the life history (two months for *G. coulteri*, MacIer and West, 1987).

In the current study it was noted that both gametophytic and tetrasporophytic phases of *Gelidium vagum* are abundant enough within the species' restricted distribution (see Chapter 3) to suggest a *Polysiphonia*-type life history. Gametophytes of the other local species, however, are rare or absent in the study area, suggesting that a *Polysiphonia*-type life history may not commonly occur in the study area, even though the species may demonstrate it under different environmental conditions or geographical areas. Instead, populations may

be propagating or persisting vegetatively, undergoing an asexual life history or repeating tetrasporophytes. In the study area, gametophytes of *G. purpurascens* rarely are found and have been collected from only a few restricted sites. Male gametophytes are difficult to differentiate in the field, although the stichidial nature of spermatangial branchlets imposes an additional order of branching making male plants detectable with some practice. It would be expected that if cystocarpic plants are collected, male gametophytes also might be present. Despite the paucity of *G. purpurascens* gametophytes, the species is abundant and widespread in the study area, and in late summer and early fall tetrasporangial plants are common. This raises the question of the kind of life history that predominates in most *G. purpurascens* populations in British Columbia and northern Washington. Are tetraspores viable, and do they produce gametophytes and ultimately cycle sexually, or do populations cycle asexually or persist vegetatively?

Gametophytes of *G. coulteri* never have been collected in the study area, although they are known from farther south (e.g. California, Dawson, 1953). Tetrasporophytes are not often fertile, observations suggest a relatively restricted time period (August to November), and fertile plants are probably overlooked. Detection is more difficult when tetrasporophytes are only weakly fertile because tetrasporophytic branchlets of *G. coulteri* are not as distinctive as in *G. purpurascens* and *G. vagum*. Detection of tetrasporangia on *Pterocladia caloglossoides* plants is difficult because the small size of these plants (never taller than 2 cm) makes low magnification necessary for detection of fertility. When *P. caloglossoides* tetrasporophytes are fertile, tetrasporangia are abundant,

and most upright axes bear fertile sori.

B. METHODS

1. Culturing

Cystocarpic and tetrasporic *Gelidium vagum* was collected from Denman Island and Galleon Point, Hornby Island (Fig. 4) in late September, 1986. Tetrasporangial *G. purpurascens* was collected from Geer Islets and Dixon Island, both on the west coast of Vancouver Island (Fig. 2) in August 1984, Georgina Point, Mayne Island (Fig. 3) in November, 1985 and February, 1986, Orlebar Point, Gabriola Island (Fig. 3) in October and December, 1985, August and October, 1986, and from Whalebone Bay, Gabriola Island (Fig. 3) in October and November, 1986. *Gelidium coulteri* tetrasporophytes were collected from Denman Island in September, 1986 and from Orlebar Point, Gabriola Island in October, 1986. Tetrasporangial *Pterocladia caloglossoides* was collected from Georgina Point, Mayne Island, Orlebar Point, Gabriola Island and Galleon Point, Hornby Island in September, 1986.

Field collected plants were brushed to remove detritus and as many epiphytes as possible before culturing. Fertile tetrasporangial or cystocarpic stichidia were excised and placed into 2×6 cm plastic petri plates containing culture medium. In the case of infertile plants, apices were excised and placed into petri dishes. For each isolate and set of growth conditions at least two dishes were prepared. The culture medium used was half strength, modified Provasoli's enriched

seawater medium (PES) (McLachlan, 1973). Initially, full strength PES was used, but it later was found that plants grew as well and with fewer contamination problems in 1/2 strength PES. This was a particular benefit in the first stages of isolation when unialgal cultures were being started. McLachlan's enriched seawater medium (SWM, McLachlan, 1973), without soil and liver extracts, also was tried. Growth was similar to that of 1/2 PES, but algal contaminants also seemed to thrive. Enrichment nutrients were filter sterilized with a 0.45 μm millipore filter prior to mixing.

Two walk-in environmental growth chambers, one at 20°C, 16:8 (light:dark photoperiod) and 20.15 $\mu\text{Em}^{-2}\text{s}^{-1}$ and one at 10°C, 8:16 photoperiod and 16.42 $\mu\text{Em}^{-2}\text{s}^{-1}$ were used, along with a smaller Percival 1-35-L incubator set at 24°C, 12:12 photoperiod and 7.91 $\mu\text{Em}^{-2}\text{s}^{-1}$. Each isolate was cultured under all three sets of conditions. Growth conditions were maintained as constant as possible but were subject to chamber breakdowns and disturbances (e.g. opening doors during dark periods). Several growth conditions were utilized to improve the chance of completion of a gelidioid life history. Conditions were not intended to test responses of plant growth and reproduction to differing environmental factors, as temperature, daylength and irradiance varied simultaneously between growth conditions. As the chambers were a group facility, they were set at temperatures and daylengths that were most amenable to all users, conditions that would not necessarily have been chosen for optimal *Gelidium* growth or life history studies. Aeration did not promote tetraspore or gamete production and made it harder to control contamination.

After incubation of spore producing tissue for 2-5 days (depending on chamber conditions), released spores and germinated sporelings were removed with a finely drawn out pipette operated by light suction and placed into petri plates of fresh medium. The medium was changed every one to two weeks depending on contamination and, after the first one to two months, once every two months. For isolates used in life history studies the medium was changed monthly.

Tetraspores or carpospores released from isolates were removed to new medium, and the above process was repeated. When monoecious isolates produced gametes, the dishes were stirred and swirled each day to encourage fertilization. In dioecious cultures, fertile male and female gametophytes were placed together into a dish and stirred and swirled daily.

Tetraspores of *Gelidium purpurascens* and *G. vagum*, and carpospores of *G. vagum* were released readily from plants under the culture conditions. Germination was most successful from the tetraspore and carpospore-bearing plants that were collected in September. At other times, spores released readily but most failed to germinate. *Gelidium coulteri* tetraspores released and germinated less readily so that few sporelings were obtained. Several techniques with fertile tetrasporophytes of *P. caloglossoides* such as drying the plants prior to immersion for release, cutting the spore-bearing branches and putting cheese-cloth covers over dishes to reduce light intensity, were unsuccessful at releasing tetraspores. For all species apical fragments also were cultured.

2. Chromosome Counts

Chromosome counts were attempted on nuclei of undivided tetrasporangia of *Gelidium vagum* collected from Hornby Island. Squashes were made by first softening excised tetrasporangial stichidia in 4% KOH for 10 minutes. Tissue was stained with Wittmann's hematoxylin (Wittmann, 1965) for one hour, the cover slip added and tissue partly squashed. New stain was added at intervals to prevent drying out. Material was destained by drawing 45% acetic acid under the coverslip for 30-60 seconds, then rinsed with distilled water and permanently mounted in 40% Karo, and tissue was firmly squashed to spread cells thoroughly.

C. SPORE GERMINATION AND EARLY DEVELOPMENT

The pattern of spore germination, and early development of the sporeling to the stage of apical cell organization, are similar in *Gelidium coulteri*, *G. purpurascens* and *G. vagum* for both tetraspores and carpospores. The following account is of spore germination events in *G. vagum*.

The released spore is spherical with a large, stellate chloroplast and prominent nucleus (Fig. 142). It germinates by producing a small hyaline protuberance (Fig. 143), which expands (Fig. 144) into a germ tube, slightly wider and longer than the spore. The spore contents evacuate into this tube, and a wall forms cutting off the cytoplasm from the spore (Fig. 145). In most cases the evacuated spore appears completely empty, but occasionally granular debris (Figs. 146, 147) or

parts of the chloroplast remain. This pattern of spore germination, referred to as "*Gelidium*-type" germination (Chemin, 1937), is characteristic of the order Gelidiales (Papenfuss, 1966; Santelices, 1974). In some spores a germ tube did not form, and the cytoplasm divided several times within the spore wall. Later germination stages were not seen, and it is unlikely that the sporeling survived.

The sporeling divides longitudinally and unequally with a curved wall (Fig. 147), to form "concave" and "fusiform" daughter cells (after Boillot, 1963). The larger, concave daughter cell, divides several times transversely and longitudinally (to the axis established by the germ tube and first division), forming 10-14 isodiametric cells (Figs. 148, 149, 150). The most distal cell of this concave cell-derived group, opposite the spore, elongates, forming a primary attachment rhizoid (Figs. 151, 152). The rhizoid is unpigmented, thick-walled and most of the cytoplasm is contained in the tip. Adjacent distal cells may subsequently similarly elongate, forming additional rhizoids (Fig. 153) that attach the sporeling to the substratum. Similar to other workers (e.g. MacIer and West, 1987) I have found that if attached sporelings are removed from the substratum they do not reattach later. Up to four primary rhizoids may form on a single sporeling.

The smaller, fusiform daughter cell of the young sporeling divides several times transversely and longitudinally to form small, isodiametric cells (Figs. 154, 155). Two longitudinally oriented clusters of cells comprise the sporeling, each cluster originating from the concave or fusiform daughter cell and separated by the first division plane of the germ tube (Figs. 155, 156). When the entire sporeling is approximately 20-40 cells large, the most proximal cell of the fusiform

cell-derived group cuts off a shallow, hemispherical apical cell (Figs. 157, 158), adjacent to the original spore wall. Up to this point in development, the sporeling increases very little in size beyond that of the germ tube.

The uniaxial construction typical for the genus develops following sporeling apical cell formation. The sporeling increases in size, growing in the opposite direction to the primary rhizoid and past the original spore wall (Fig. 160). Organization is uniaxial and shows a characteristic pattern. The domed, hemispherical, apical cell divides transversely (Figs. 159, 162). The subapical cell divides obliquely twice to form two lateral, opposite periaxial cells. Each periaxial cell cuts off a lateral second order filament that grows outwards contributing to thallus width (Figs. 68, 159). Periaxial cells also cut off third order filaments above and below the lateral plane that determine the thickness of the thallus. As the sporeling increases in length, branch initials are formed from modified cortical cells (Fig. 159), and attachment pads develop from the outward elongation and adhesion of cortical cells (Fig. 161).

D. REPRODUCTION IN CULTURED AND FIELD PLANTS

1. Culture Results

Reproductive structures appeared on cultured plants of *G. vagum*, when they were one to two cm tall, approximately 5-6 weeks after spore germination in isolates grown at 20°C. Cultured tetrasporophytes, with the same morphology as tetrasporophytes from the field, produced tetrasporangia on stichidia (Figs. 163,

166). Plants grew faster at 20°C and high light (long day and high irradiance) and were narrower and less flattened than field collected plants. At 24°C and low irradiance, plants were narrow and weak and only sparsely fertile. Tetrasporophytes grown at 10°C grew more slowly and took much longer (several months to one year longer) to become fertile, if they did so at all. Plants grown at 10°C more often appear similar to field material, with wider, flatter branches than plants grown at 20°C. Tetrasporangial stichidia were determinate, cylindrical, with rounded or acute apices and produced tetrasporangia around the entire stichidium in an unordered manner. Tetraspores were released and germinated into gametophytes (Fig. 167).

Gelidium vagum gametophytes produced carpogonia and spermatangia on short branchlets (Fig. 164), as in field collected material, with an axis producing a succession of fertile branchlets so that progressively older carpogonia and post-fertilization stages were observed in more proximal parts of the plant (Fig. 165). Developmental sequences of male and female gametes and carposporophytes were similar to those observed in field collected gametophytes. Fertilization occurred in culture, although not as extensively as in field material, evidenced by the lower frequency of cystocarps per cultured plant. Greater water motion in the field than in the occasionally swirled, unaerated cultures may bring non-motile spermatia in contact with trichogynes more effectively. After three to five weeks, carpospores were released from bilocular cystocarps. Carpospores were obpyriform when in the cystocarp, but rounded-up on release. They contained a large, central, stellate chloroplast and germinated into tetrasporophytes, as previously described.

Gelidium vagum isolates originally initiated from tetraspores and carpospores, produced fertile gametophytes and tetrasporophytes (respectively), that in turn released carpospores, and tetraspores, and germinated into tetrasporophytes and gametophytes. These tetrasporophytes (Fig. 168) and gametophytes became fertile completing the life history (simultaneously from two different starting points).

Life histories could not be completed in culture for the other local gelidiaceous species, but some insights into the life histories of *Gelidium purpurascens* and *G. coulteri* in the study area were provided by culturing. Tetrasporangial stichidia of *Pterocladia caloglossoides* were placed into culture several times, but tetraspores were not released under any of the experimental culture conditions, and the vegetative fragments grew well at 10°C and 20°C but did not become fertile.

Cultures of *G. purpurascens* were started from tetraspores released from field-collected tetrasporophytes (Fig. 169). The resulting gametophyte sporelings were dioecious; both carpogonial and spermatangial plants were readily identifiable in the cultures grown at 20°C. Fertile male gametophytes developed in four isolates (Geer Islets, Dixon Island, Mayne Island and Whalebone Bay on Gabriola Island, Fig. 170). Female gametophytes were identified only in Whalebone Bay cultures (Fig. 171). Carpogonia and spermatangia in cultured plants appeared mature but evidence of fertilization was not observed, possibly due to inadequate water motion in culture dishes, so that non-motile spermatia could not contact trichogynes. Alternatively, fertilization might have occurred but gamete incompatibility or inviability, or early carposporophyte abortion could make fertilization undetectable.

Tetraspores did not release as readily from stichidia of *Gelidium coulteri*, and germination was less successful than for *G. purpurascens* and *G. vagum*, but a few isolates of gametophytes were maintained. Fertile gametophytes (found only in 20°C cultures) were male (Figs. 19, 172, 173); female gametophytes were undetected (under all growth conditions), and it is unknown if they existed in the cultures, or if all non-fertile plants from tetraspores were male gametophytes or simply non-reproductive.

Undivided tetrasporangia were used for chromosome counts rather than other cell types (e.g. apical cells, spermatangia or spermatia) because they had the largest nuclei and most visible chromosomes. Even so, nuclei were still small (3-7 μm diameter), and it was difficult to obtain the resolution necessary for reliable chromosome counts. Counts were made only on nuclei squashed flat enough so that chromosomes were in a single focal plane. Counts of 14 bivalents and a single, additional small body were made on the best view of a dividing nucleus (Figs. 174, 175). The bivalents consisted of closely paired chromosomes at the most contracted stage of prophase I. It was unclear whether the small body was a separate chromosome, small bivalent or an extraneous body. If it was a small chromosome, then it should be noted that there are size differences between some chromosomes.

Counts of 14 and at least 12 and 13 (Fig. 176) bivalents were obtained from other dividing tetrasporangial nuclei. Nuclei in which counts of 12 and 13 bivalents were obtained contained a large deeply staining body probably comprising two or more bivalents. Thus the chromosome number obtained from

all of these counts is $n=14-15$. In some nuclei, five large bodies were observed (Fig. 177).

2. Field Observations of Reproduction

Tetrasporophytes and gametophytes of *Gelidium vagum* are equally abundant in the field when reproductive plants were found (August to October). Gametophytes of *G. purpurascens* were rarely collected in the study area. Of 18 sites in the study area from which I collected *G. purpurascens*, female gametophytes were found at only two sites (Kirby Point, Diana Island and Geer Islets, both in Barkley Sound). Six specimens of *G. purpurascens* female gametophytes were found from a total of several hundred records of this species (UBC A41842, Fleming Island, Barkley Sound; UBC A39126, Lawn Point, Vancouver Island; UBC A41586, Wizard Island, Barkley Sound; UBC A36876, Amos Island, west coast of Vancouver Island; UBC A41946, Clarke Island, Barkley Sound; and UBC A46026, Bamfield, Barkley Sound; UBC A67446, Kirby Point, Diana Island). Male gametophytes of *G. purpurascens* rarely are collected from the study area, and the only specimens known are three records in UBC (UBC A53657, Nootka Sound, Vancouver Island; UBC A10813, Esteban Point, Vancouver Island; and UBC A41415, Tzartus Island, Barkley Sound).

Tetrasporophytes of *Gelidium purpurascens* are much more abundant than gametophytes in my own collections and in herbarium specimens. Whereas *G. purpurascens* gametophytes were found only in the southern half of the study area, while tetrasporophytes were found fertile throughout British Columbia and

to its northern range limit in southeast Alaska (UBC A69432). Where female gametophytes occur, they bear many fully developed cystocarps, suggesting that successful fertilization occurs in the field. Tetrasporangia are abundant and appear deeply pigmented.

Fertile gametophytes of *Gelidium coulteri* are unknown in the study area, but occur farther south in California. Although tetrasporangial specimens have been collected in British Columbia, they are not often abundantly fertile. Gametophytes also are unknown for *Pterocladia caloglossoides* from the study area, but are reported for this species from California (Dawson, 1953). *Pterocladia caloglossoides* is tetrasporophytic throughout British Columbia, but infertile in southeast Alaska collections (UBC A69423, UBC A69424, UBC A69425, UBC A69426, UBC A69427, UBC A69428, UBC A69429, UBC A69430, UBC A69431).

E. DISCUSSION

1. Spore Germination

Spore germination of *Gelidium vagum*, *G. purpurascens* and *G. coulteri* is uniform for all types of spores and the species. In this study, it was impossible to differentiate species or spore types using any aspect of spore morphology or germination pattern. The similarity between tetraspore and carpospore germination also was noted by Yamasaki (1960). Furthermore, spore germination in *Gelidium* (Chemin, 1937; Katada, 1949, 1955; Yamasaki, 1960; Boillot, 1963; Kaneko, 1966; Guzman-del Proo *et al.*, 1972; this study), *Pterocladia*, *Acanthopeltis*

(Katada, 1955) and *Gelidiella* (Chihara and Kamura, 1963; Sreenivasa Rao, 1971) showed the same general pattern.

Differences in reports of spore germination are minor and, in at least some instances, appear to result from the interpretation of observations. For several hours after release, and before attachment, carpospores of some *Gelidium* species reportedly showed amoeboid motion (Boillot, 1963; Guzman-del Proo *et al.*, 1972). Chemin (1937) in particular was fascinated by carpospore mobility, reporting paths and rates for spores of several red algal species. Germination observations of the local species of *Gelidium* were started after spore attachment, thus amoeboid motion, if present, was not observed in the spores. Several authors observed one to three (mitotic) nuclear divisions before spore evacuation in *G. vagum* (Kaneko, 1966), *Gelidiella acerosa* (Sreenivasa Rao, 1971), *Gelidium latifolium*, *G. pulchellum* and *G. pusillum* (Boillot, 1963), but only a single nucleus entered the germ tube.

Empty spore germination (i.e. evacuation of the entire spore cytoplasm into the germ tube) occurs in other higher rhodophyte groups, but only in Gelidiales is it followed by direct development of the upright plants, without an intervening discoid phase. Chemin (1937) designated all species with empty spore germination, as having "*Gelidium*-type" germination regardless of subsequent developments, and Boillot (1963) later demonstrated the unique nature of this pattern in *Gelidium*.

The first division of the germ tube after being cut off from the spore, is highly significant, as the plane of this division and the distinctive concave and fusiform

daughter cells determine the polarity of the sporeling. Observations of the sporeling from different orientations can be misleading. For example, Sreenivasa Rao (1971) noted that the first division of the germ tube in *Gelidiella acerosa* is sometimes transverse. This interpretation probably resulted from a face view of the fusiform or concave cell (analogous to a valve view of a diatom), where the curved wall separating them is not seen, and the first divisions of fusiform and concave cells is transverse. There appears to be general agreement that the concave cell is the larger of the two daughter cells and gives rise to the primary rhizoid (Boillot, 1963; Chihara and Kamura, 1963; this work). However, there is confusion in the literature as to the developmental role played by the fusiform and concave cells. In *Gelidiella acerosa*, Sreenivasa Rao (1971) reported that the rhizoid originated from the fusiform cell group, whereas Chihara and Kamura (1963) observed the opposite, a concave cell group origin of the rhizoid. It is most likely that the concave cell produces the primary rhizoid in *Gelidiella* as in the other members of the order. In a series of manipulations of the environment of germinating spores, Katada (1949, 1955) determined that the specific gravity of the water affected the length and number of rhizoids produced, but the pattern of development was invariant.

Interpretation of the origin of the apical cell also has caused confusion. Observations made here indicate that the fusiform cell group is the site of apical cell production. Specifically, the cell of the fusiform cell group closest to the spore wall, differentiates as the apical cell. This agrees with the pattern illustrated or stated by some workers in *Gelidium amansii*, *G. divaricatum*, *G. japonicum*, *G. pacificum*, *G. pusillum*, *Pterocladia tenuis* and *Acanthopeltis japonica*.

(Katada, 1955), *G. amansii* (Yamasaki, 1960), and *Gelidiella acerosa* (Chihara and Kamura, 1963) but is contrary to observations of Boillot (1963). Boillot (1963) reported the formation of two apical initials in *Gelidium latifolium*, *G. pulchellum* and *G. pusillum*, one contributed by the fusiform cell group and another from the concave cell group. It was unclear which initial takes over, but Boillot believed that it is the apical cell from the concave cell group. Yamasaki (1960) illustrated a developmental sequence like that observed in the local *Gelidium* species, but he referred indistinctly to "upper" and "lower" daughter cells. He stated that the upper daughter cell gave rise to the creeping part of the plant, and the lower daughter cell produced the primary rhizoid and upright or creeping branches. This is unlike any other gelidioid developmental pattern; it may correspond to the dual apical cells mentioned by Boillot. According to Yamasaki's (1960) illustrations, however, spore development patterns appear similar to those of other gelidioids. Some authors do not indicate the origin of the apical cell, and their illustrations are sometimes too confusing to determine where this occurs (e.g. Kaneko, 1966; Sreenivasa Rao, 1971).

In conclusion, possibly the most notable feature of spore germination and early sporeling development in Gelidiales is the uniformity of the developmental pattern in all species, genera and spore types studied to date, and the distinctiveness this pattern with respect to other red algal groups.

2. Life Histories

The completion of the *Gelidium vagum* life history in culture shows that the British Columbian plants have a *Polysiphonia*-type life history and, based on the common occurrence of tetrasporophytes and gametophytes in the field, this seems to be an accurate description of the life history in the study area.

The scarcity with which *Gelidium purpurascens* gametophytes are collected in the study area suggests that gametophytes either fail to survive in the field (for example, if environmental conditions are unsuitable for spore or sporeling survival or full development) or gametophytes are commonly produced but rarely become fertile (and hence are not recognized as gametophytes). Low abundance of fertile male and female gametophytes in the field would decrease the chance of fertilization, accounting for the rarity of cystocarpic *G. purpurascens* in the study area. In British Columbia, *G. purpurascens* is probably capable of completing the *Polysiphonia*-type life history, but factors such as low tetraspore viability and low abundance of gametophytes or unfavourable environmental conditions could prevent the common occurrence of this sexual life history *in situ*. Occasional periods of favourable environmental conditions could permit the completion of a sexual *Polysiphonia*-type life history and range expansion, whereas more commonly, populations persist vegetatively or reproduce asexually.

The British Columbian populations of *Gelidium purpurascens* persist by perennating as upright fronds or creeping axes and may recruit new individuals when environmental conditions are suitable for gametophytic sporeling survival or

gametophyte fertility. Drift plants of *G. purpurascens* appear to be whole plants torn from the substratum rather than fragments of plants. Drift plants and attached plants in the field do not exhibit the abundant production of attachment pads that can be seen in cultured plant fragments. These observations suggest that propagation by vegetative fragmentation does not appear to be a mechanism operating here, but further field experimentation would be necessary for confirmation. Although there was no evidence in cultures, tetraspores also could be recycling apomictically as alternate dispersal agents. However, further work would be required to determine if such a process is operating in British Columbia *G. purpurascens*. Information on whether populations are expanding or simply maintaining their current extent would also be needed to assess the existence and importance of the above mechanisms.

The occurrence of fertile male *Gelidium coulteri* plants in 20°C culture was significant because, although none were recorded from the study area (from herbarium records or my own collections), their presence indicates that the capacity for the production of male gametophytes exists. Male plants could be overlooked in the field or they could be absent if environmental conditions are not suitable for their development. A seawater temperature of 20°C is uncommon in British Columbia but is occasionally reached, particularly in sheltered locations. *Gelidium coulteri* probably propagates vegetatively by creeping axes and possibly apomictically by tetraspores in British Columbia, at the northern limit of its distribution.

In culture, *Pterocladia caloglossoides* failed to release (or germinate *in situ*)

tetraspores, although they were abundant on stichidia. It is unknown if tetraspores function at all as propagules (meiotically or apomictically). Clearly vegetative propagation and perennation by creeping axes must be important in the maintenance or expansion of this species' local populations.

Gelidium purpurascens, *G. coulteri* and *Pterocladia caloglossoides* display the pattern of latitudinal variation in life history expression proposed by Dixon, (1965), where progressively more northern populations of a species show reduced fertility of gametophytes and, even more northerly populations show reduced tetrasporophyte fertility. This trend ultimately results in only vegetative propagation at species' northern range limits. *Gelidium purpurascens* varies in part from this pattern because some of the most northerly collections of this species, from southeast Alaska, are tetrasporangial (UBC A69432).

3. Chromosome Counts

There have been few chromosome counts made on any Gelidiaceae. Kaneko (1966) observed $n=7-10$ in tetraspore germlings of *G. vagum*, Dixon (1954) obtained $n=4$ and 5 for cells of the cortex and nutritive tissue of female gametophytes of *G. corneum*, and Kaneko (1968) counted $n=15$ for tetrasporangia of *Acanthopeltis japonica*. A demonstration of the uncertainty often associated with such counts is offered in the observations made on *G. latifolium*. Dixon (1954) obtained "4, 5, 9 and 10 chromosomes" for carposporophytes, but Boillot (1963) obtained $n=18$ for young gametophyte sporelings, and Magne (1964) counted n or $2n=25-30$ for vegetative cells of *G. latifolium* var. *luxurians*. It is possible that

some varieties could represent polyploid groups (e.g. is *G. latifolium* var. *luxurians* a polyploid of *G. latifolium* var. *latifolium*?) or that polyploids could be included with diploids in the same, or other, species. Counts of $n=14-15$ obtained from undivided tetrasporangia of *G. vagum* in this study, are closest to counts made by Boillot (1963) for *G. latifolium* and by Kaneko (1968) for *Acanthopeltis japonica*. The *G. vagum* counts of $n=14-15$ made here are not similar to and do not appear to be multiples of the only other counts made for *G. vagum* ($n=7-10$) by Kaneko (1966). The importance of good preparations and accurate information about cell type and generation counted must be stressed. Too few reliable studies have been made and corroborated to confidently state chromosome numbers of species, genera or base numbers for the family.

CHAPTER V. CARPOGONIUM AND CARPOSPOROPHYTE DEVELOPMENT

A. INTRODUCTION

As with spermatogenesis and tetrasporogenesis, there have been few detailed studies on pre- and post- fertilization development in *Gelidium*. The most recent investigations were those of Dixon (1959) on *G. latifolium* and *G. pulchellum*, Fan (1961) on *G. robustum* (as *G. cartilagineum* var. *robustum*) and Hommersand and Fredericq (1988) on *G. pteridifolium*. Additional selected species of genera of Gelidiales, *Gelidium*, *Pterocladia*, *Suhria*, *Beckerella*, and *Acanthopeltis*, have been observed for comparative purposes (Fan, 1961; Hommersand and Fredericq, 1988) or briefly touched on in other studies (Kraft, 1976). The interpretations made by Hommersand and Fredericq (1988) were significantly different from previous authors, most notably as to the nature of the carpogonium, and their findings were corroborated in the current investigation. In view of the paucity of studies on these developmental processes, additional descriptive observations based on local *Gelidium* species were warranted.

The lack of studies of pre- and post- fertilization development in members of Gelidiales is surprising given the healthy debate that has taken place historically over the ordinal recognition of the group. Gelidiales now are recognized almost universally at the ordinal level by a suite of characters other than female reproductive and carposporophyte characters, such as a single pit plug cap, *Gelidium*-type spore germination, the presence of rhizines, the production of two distichous periaxial cells, and the transverse division of the spermatangial mother

cell in forming a spermatangium (see Chapter 1, herein; Santelices, 1974; Gabrielson and Garbary, 1986, 1987; Hommersand and Fredericq, 1988).

Kylin (1923) elevated the Gelidiaceae, a family in the Nemaliales (as "Nemalionales"), to ordinal status based on the unchanged, fertilized carpoponium that was the starting point for the gonimoblast, and auxiliary cells that are present but function as nurse cells in nutrition of the carposporophyte. Kylin's (1923) system of ordinal classification, was based entirely on features of the female gametophytic and carposporophytic reproductive apparatus, namely characters of the carpoponial branch, auxiliary cells, connecting filaments and the gonimoblast. It must be noted that contrary to Dixon's (1961) belief that life history differences (Kylin, 1923 believed Nemaliales were haplobiontic and Gelidiales diplobiontic) were also a criterion in the elevation of Gelidiaceae to an order, Kylin (1923) discussed these differences but stated that the life history character should not be used systematically at that time. Both Nemaliales and Gelidiales lacked a "typical" auxiliary cell, i.e. any cell that the carpoponium fused with and from which the gonimoblast developed (Kylin, 1928, 1956), and thus were set apart from the other orders (Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales) which possessed a "typical" auxiliary cell. Kylin (1928) studied pre- and post-fertilization events in detail in Gelidiaceae, and the family was important in his formulation of the distinction between "generative" ("typical") and "nutritive" auxiliary cells. Even after his addition of the criterion that a typical auxiliary cell also not be a carpoponial branch cell (Kylin, 1935, 1937), he still maintained that a "typical" auxiliary cell was absent in Gelidiales. Kylin (1928) believed that the gonimoblast in Gelidiales developed directly from

the unchanged, fertilized carpogonium. When this was found to be erroneous and that the carpogonium underwent non-obligate fusions with adjacent cells (Dixon, 1959), the presence of generative auxiliary cells became open to interpretation and dependent on the definition of the auxiliary cell. Drew (1954) gave the most useful definition, as a cell of specified position in the thallus with which the carpogonium fuses prior to gonimoblast formation. Accordingly, Gelidiales lack an auxiliary cell. Details of the auxiliary cell debate are given by Santelices (1974) and Hommersand and Fredericq (1988). The ordinal position of Gelidiales has been questioned extensively (Dixon, 1959, 1961; Papenfuss, 1966), being largely dependent on the perceived presence or absence of an auxiliary cell and, in Dixon's (1961) view, the lack of life history differences, i.e. haplobiontic Nemaliales (as Nemalionales) and diplobiontic Gelidiales. Dixon (1961) argued for returning Gelidiales to Nemaliales as Kylin's (1923, 1928, 1956) characters separating the orders were not valid. On the other hand, Papenfuss (1966) argued that Gelidiales was supported at ordinal rank by the presence of unique chains or nutritive auxiliary cells, by the presence of only two periaxial cells, and by the unique pattern of spore germination. It is important that carpogonium and carposporophyte development be studied in more gelidioids to assess variation at the genus and species levels and to determine features of the developmental processes that are common to all members of the order. The present study contributes to a more complete understanding of carpogonium and carposporophyte development in the order.

B. METHODS

Of the local gelidiaceous species, fertile (female) gametophytes of only *Gelidium vagum* and *G. purpurascens* occurred in the study area. Carposporophyte development followed the same pattern in both species. The process also was observed, in less detail, in sections from herbarium specimens of *G. robustum* (UC 395419, 647822, 756470, 940173; UBC A62199).

Observations were made on dried and Formalin preserved material following the light microscope methods described in Chapter 3. Hematoxylin stained material also was used, prepared as in Chapter 4. The only modification for the purpose at hand (as opposed to chromosome counts) was to make light squashes of apices and cystocarps, enough to spread out clusters of filaments, but not enough to break the continuity of filaments. Hand made razor blade sections were stained with hematoxylin for 1 h, destained with 45% acetic acid for 15-30 s, and rinsed with water.

C. CARPOGONIUM DEVELOPMENT IN GELIDIUM

The carposporophyte of *Gelidium* differentiates from the second to basal cell of a third order filament that is a normal part of the vegetative thallus. In the vegetative condition, the second to basal cell of a third order filament is intercalary with three pit-connections: one to the basal cell of the filament, one to the third cell of the filament, and one to another distal cell basal in a fourth order filament. When differentiated as a carposporophyte, the second to basal cell of

a third order filament bears only two pit-connections (Fig. 182), one to the basal cell and one to the third cell of the third order filament. The fourth order vegetative filament and any of its higher order branches, is replaced by the gradually tapering, arched trichogyne of the carpogonium. Carpogonia are produced sequentially along the axis in the fertile area (Fig. 47). Two rows of carpogonia are produced lateral to the axial row, on third order filaments, extending to both surfaces of the compressed to flattened axis. Thus four rows of carpogonia are formed, with two rows visible in either a longitudinal or saggital section. Replacement of a cluster of vegetative cortical cells by the trichogyne of each carpogonium results in the production of a medial gap or furrow on both thallus surfaces (Fig. 89). Trichogynes protrude to the thallus surface through this gap.

The carpogonium is initially intercalary. Farther back in the fertile zone are sessile carpogonia, cut off from the supporting cell by a distinctive concave wall (Fig. 179). These carpogonia have a single pit-connection to an intercalary supporting cell, and a second to basal cell in a third order filament. The question arises whether intercalary carpogonia are immature, and require a final division to become mature and sessile, or whether intercalary carpogonia are mature and functional, and undergo a division that discards the trichogyne after being past receptivity to spermatia. Many sessile carpogonia were observed in a degenerating condition, whereas the supporting cell appeared healthy (i.e. cytoplasm dense as in adjacent cells) (Fig. 180). An intercalary carpogonium, just fertilized, was observed at an early stage of fusion cell initiation, fusing to cortical cells through two expanded pit-connections (Fig. 181). Thus functional carpogonia are intercalary in *Gelidium*.

D. CARPOSPOROPHYTE AND CYSTOCARP DEVELOPMENT

Following fertilization, the carpogonium expands and fuses with several adjacent cells through widened pit-connections, forming a large, irregularly lobed, multinucleate fusion cell (Figs. 182, 183). Occasionally two fusion cells can be seen in a single apex, suggesting that two carpogonia may be fertilized and develop a genetically heterogenous carposporophyte in a single cystocarp. It is difficult to be certain of this, however, as fusion cells may have very long, narrow lobes. When the fusion cells are widely spaced it seems likely that the carposporophytes are discrete.

Concomitant with fertilization, short chains of small, isodiametric cells, referred to as "nutritive filaments" (Hommersand and Fredericq, 1988), are cut off from the bases of cells of third order filaments (Figs. 183, 184). In *Gelidium vagum*, fusion cells always are present in apices where nutritive filaments are seen, suggesting that nutritive filaments are initiated at fertilization. The timing of nutritive filament formation may be slightly earlier in *G. purpurascens*, as nutritive filaments are present in apices that appeared to lack a fusion cell. Nutritive filaments curve in, towards and around the plate of second order cells (Fig. 183). They reach a maximum length of six cells, the apical cell of which is slightly larger and round, other cells being short and cylindrical (Fig. 184). All nutritive filament cells have prominent nuclei.

Fusion cell lobes cut off weakly staining, uninucleate gonimoblast cells, which form branching chains winding around and between cells of the second order

plate (Fig. 185). Gonimoblast cells cut off elongated processes that contact and fuse with apical cells of nutritive filaments (Fig. 186). This fusion is between the diploid gonimoblast (carposporophyte) cells and haploid nutritive filament (female gametophyte) cells.

The gonimoblast produces single carposporangia laterally and terminally (Fig. 188), which project into one of the two locules created between the second order plate and cortex at cystocarp expansion (Fig. 187). Uninucleate carposporangia expand to become ovate to obpyriform with a darkly staining cytoplasm and stellate chloroplast. Carposporangia are produced continually by the gonimoblast, and a variety of ages is seen in a cystocarp. The growing carposporophyte pushes the cortex away from the second order plate, creating locules and causing third order filament cells to stretch. In *Gelidium vagum* these filaments break as the cystocarp matures (Fig. 187), whereas they remain intact in *G. purpurascens* (Fig. 50). The cystocarp cortex does not increase in thickness over the vegetative cortex, although some cells stretch laterally.

At maturity the cystocarp has expanded beyond the width of the vegetative branch. Locules are filled with carposporangia and faintly staining mucilage (Fig. 187). Nutritive filaments no longer are distinguishable, and the entire carposporophyte (except the sporangia) appears vacuolate, staining poorly (with aniline blue). One ostiole per locule forms in the cystocarp cortex, due to the failure of part of the cortex to fill in. It is a simple, round opening and is not beaked or protruding. There is no evidence that the ostiole forms from tearing of the cortex. Carpospores are released, leaving behind some mucilage in the

cystocarp. There is no evidence of percurrent production by carposporangia.

E. DISCUSSION

The most significant observation made concerns the intercalary carpogonium in *Gelidium*. Dixon (1959) and Fan (1961) both noticed intercalary carpogonia but their interpretations differed. Intercalary carpogonia were interpreted as early developmental stages by Fan and as aberrant gametes by Dixon. They both agreed that intercalary carpogonia are non-functional. They saw sessile carpogonia as mature, but no basis for this assumption was provided, on the other hand, Hommersand and Fredericq (1988) regarded intercalary carpogonia as functional and sessile carpogonia as non-functional; while they examined the material no illustrations were provided.

In this study on *Gelidium*, an intercalary carpogonium was observed fusing with cortical cells through two widened pit-connections at a very early stage of fusion cell formation. Sessile carpogonia never formed fusion cells and often were seen in stages of degeneration, suggesting a non-functional condition. Reports of sessile carpogonia fusing with the supporting cell following fertilization (Dixon, 1959; Fan, 1961), stem from observations of fusion cells where the outline of the carpogonium is visible, and where the fusion cell narrows somewhat before expanding at what is believed to be the supporting cell. Early stages of this supposed fusion are not reported or illustrated. In *G. vagum* and *G. purpurascens*, however, intercalary carpogonia are expanded at the base, particularly near pit-connections. Hommersand and Fredericq (1988) reported the

retention of part of the carpogonium outline in the fusion cell, and this could explain the earlier reports of Fan (1961) and Dixon (1959), if the basal carpogonium outline is retained as well as the trichogyne lobe. It should be noted that fusion cells in *G. vagum* and *G. purpurascens* showed no carpogonial outline, but this observation probably depends on fusion cell age. Thus the functional carpogonia of *Gelidium* are intercalary, as noted by Hommersand and Fredericq (1988). If unfertilized, the carpogonium can cut off the trichogyne lobe, and the basal portion can then revert to a vegetative cortical cell.

An apical notch is reported in female branchlets of *Gelidium robustum* (Fan, 1961) and *G. pteridifolium* (Hommersand and Fredericq, 1988), but is absent in British Columbia *G. purpurascens* and *G. vagum*. Hommersand and Fredericq (1988) speculated that retarded growth of the apical cell and axial row, evidenced by overgrowth of the apical cell by adjacent cortical lobes, was responsible for development of the axial furrow. Growth of third and higher order filaments near the axial row was outpaced by equivalent filaments lateral and distal to the axial row. Since there does not appear to be retarded axial row development in species lacking an apical notch, the axial furrow was interpreted as originating from the absence of cortical cells that belonged to filaments replaced by the carpogonium.

Timing of the formation of nutritive filaments varied between studies and may be a taxonomically useful character. In *Gelidium pteridifolium* nutritive filaments are initiated as the carpogonium differentiates, but before fertilization (Hommersand and Fredericq, 1988). In *G. latifolium*, and *G. pulchellum* (Dixon,

1959), *G. robustum* (Fan, 1961) and *G. vagum* (herein), fertilization is probably the stimulus for nutritive filament production. It is difficult to be certain from reports by Dixon and Fan, as both believed the functional carpogonium was intercalary. Thus when Dixon (1959) observed that nutritive filaments formed after the carpogonium was mature, the observation corresponds to filaments forming after the carpogonium became sessile and non-functional, while other functional carpogonia probably were being fertilized. Fan (1961) noted that nutritive filaments form during carpogonium development, but the exact timing is not known with respect to functional carpogonia or fusion cell formation.

While there is general agreement that, upon fertilization, the carpogonium enlarges and becomes lobed, there is no agreement on the nature of fusions with surrounding cortical cells. Fan (1961) reported no fusions between the fusion cell and cortical cells, whereas Dixon (1959) accepted that fusions may occur but are not obligate. In this study and in all fusion cells seen by Hommersand and Fredericq (1988) there were fusions with cortical cells. My findings agree with Hommersand and Fredericq (1988) that fusions of the carpogonium with cortical cells are a regular feature of early carposporophyte development in *Gelidium*, but that the fusions may not be with cells in a specified position with respect to the carpogonium.

Fusions regularly occur between cells of nutritive filaments and gonimoblast cells. Nutritive filament cells clearly function as nutritive auxiliary cells, as their initially dense cytoplasm becomes sparse and vacuolate after fusions and gonimoblast growth. I suspect that the reported lack of fusions between nutritive

filaments and gonimoblast (Dixon, 1959) resulted simply from failure to observe them and not their absence. Confirmation should be made, as substantiation of Dixon's (1959) observation could be important systematically.

Gelidium latifolium and *G. pulchellum* differ from *G. purpurascens* and *G. vagum* in carposporangial initiation. In the former two species, clusters of uninucleate initials are cut off from gonimoblast cells that develop into carposporangia (Dixon, 1959). In the British Columbian *Gelidium* species, carposporangia are produced singly, terminally and laterally on gonimoblast cells, rather than in clusters. In *G. latifolium* and *G. pulchellum*, the cystocarp cortex is thicker than the vegetative cortex and ostioles do not form; carpospores are released by degeneration of the pericarp (Dixon, 1959). This is different from *G. purpurascens* and *G. vagum* where the cystocarp cortex is not thickened over the vegetative cortex and one ostiole is present per locule. In *G. pteridifolium* the "potential ostiolar regions" that develop have plugs that later break down allowing carpospore release (Hommersand and Fredericq, 1988).

Returning briefly to the question of the presence of an (generative) auxiliary cell in Gelidiales, discussion of this problem is given by Hommersand and Fredericq (1988). It is clear that the gonimoblast develops from lobes of the fusion cell and not from an unchanged carpogonium as Kylin (1928) proposed. Having shown in this work that the functional carpogonium is intercalary and not sessile, observations interpreted as carpogonia fusing with their supporting cells are erroneous and are actually of the unequal enlargement of some parts of the carpogonium (e.g. base and pit-connection region) relative to others. Using Drew's

(1954) definition of an auxiliary cell, the possibility that the supporting cell functions as an auxiliary cell can be eliminated, as a supporting cell does not exist in *Gelidium*. Given that fusions with cortical cells occur, the question of the nature of the auxiliary cell is reduced to whether any particular cortical cell is always the cell with which the carpogonium fuses and whether it functions in initiation of the gonimoblast or is strictly nutritive. I concur with Hommersand and Fredericq (1988) that there is, as yet, too little information available on cortical cell specificity or function. Recent observations do not suggest that a specified cortical cell is involved, and gonimoblast was not seen developing from the part of the fusion cell that fused with cortical cells. In light of Hommersand and Fredericq's (1988) findings, and because some previously reported developmental patterns have been generalized as "typical for the Gelidiales" (e.g. Kraft, 1976), re-investigations of previously studied species would be in order to clarify debated aspects.

CHAPTER VI. SPERMATIOGENESIS IN *GELIDIUM*

A. INTRODUCTION

In 1925, Grubb wrote the following concerning the state of knowledge of male organs in the Florideae (Grubb, 1925).

Although nearly 150 years have passed since the first record of spermatia in the red algae occurred in print, our knowledge of these minute bodies which play so important a part in the reproductive processes of Rhodophyceae is surprisingly inadequate and limited. Records of the observation of antheridia in more than 120 European species of the Florideae are to be found scattered in algal literature, but of these the vast majority are simply notes to the effect that male plants have been seen and recognized. A certain number give a short description detailing the position of the antheridia, and some include a slight account of their structure, but owing to the nature of the material, modern cytological methods of investigation have only been brought to bear on a few forms.

A similar situation still exists in 1988, even though spermatangia are known for many more species, and their development at the light microscope level has been studied to some extent. Few observations of the development of red algal male gametes have been made using electron microscope techniques.

The male gamete and associated structures generally do not play an important role in red algal taxonomy. The presence of spermatangia and their position on the thallus are noted, but spermatangial morphology usually is not used as a taxonomic character. Part of the reason for this is the often cryptic nature of male gametangia and the resulting rare collections of male gametophytes or their recognition as such in collections. The importance of male characters in taxonomy

also depends on the taxonomic rank at which the characters can be applied. Gabrielson and Garbary (1987) recently have used spermatangial characters at the ordinal rank in the construction of phylogenetic trees. For example, the transverse (as opposed to oblique) division of the spermatangial mother cell to form a spermatangium is characteristic of Gelidiales (Gabrielson and Garbary, 1987). At the generic rank, spermatangial position has been used to separate *Gracilaria* into subgenera (Yamamoto, 1975) and to differentiate the genera *Gracilaria* and *Polycavernosa* (Bangmei and Abbott, 1985). As has been discussed earlier in this work, the character of spermatangia occurring on the same plants as carpogonia (i.e. monoecy) instead of on separate male gametophytes (i.e. dioecy) can be used at the specific rank to recognize *Gelidium vagum* and *G. johnstonii*.

Spermatangia are known for approximately 30-40% of gelidiaceous species, but their development seldom has been followed (Dixon, 1959; Akatsuka, 1970, 1973; Tazawa, 1975). This is understandable given the small size of spermatangial mother cells, spermatangia and spermatia.

Among the nine electron microscope studies of male gamete development in higher rhodophytes, none has been carried out on a member of the Gelidiales. Electron microscopy of male gametes was performed primarily on ceramialean genera (Peyrière, 1971, *Griffithsia flosculosa*; Kugrens and West, 1972b, *Levringiella gardneri* and *Erythrocytis saccata*; Scott and Dixon, 1973a, *Ptilota densa*; Kugrens, 1974, *Janczewskia gardneri*; Scott et al., 1980, *Polysiphonia denudata* and *P. harveyi*; Kugrens, 1980, *Polysiphonia hendryi*), and *Bonnemaisonia*

hamifera (Bonnemaisoniales) also was studied (Simon-Bichard-Brèaud, 1971, 1972a, 1972b). In addition, there are some fragmentary reports on other higher rhodophytes (Peyrière, 1974, *Polysiphonia*, *Rhodomela*, *Laurencia*, *Polyneura*, *Nitophyllum*, and *Furcellaria*). In this study spermatogenesis in two of the four gelidioids was followed using light and electron microscopy, as well as in a third species using only light microscopy.

B. METHODS

The species used in this study were *Gelidium purpurascens*, *G. vagum* and *G. coulteri* (light microscopy only). Light microscopy methods are included in Chapter 3. Both field and cultured material of *G. vagum* was fixed for electron microscopy. *Gelidium purpurascens* male gametophytes were not seen in the field, consequently material for this study on spermatogenesis was obtained from male gametophytes grown to maturity in culture from tetraspores.

Freshly collected field material was acclimated to a constant photoperiodic regime (12:8 LD in 20°C) in order to synchronize cell divisions with light and dark periods. Material was fixed 1 to 2 h after chamber lights came on to maximize the chance of seeing cell division (van der Meer, pers. comm.). Fixation was carried out in the refrigerator for 7 h in 2.5% glutaraldehyde, a 1:1 mixture of 5% glutaraldehyde and Sorensen's phosphate buffer (pH 7.2). For rapid fixation and to prevent blockage of intercellular spaces by air bubbles, tissue was cut from plants in a drop of fixative. Fixed tissue was postfixed in 1% osmium tetroxide in the refrigerator for 16 h and then dehydrated. Dehydration was

carried out in a graded methanol series. Material was infiltrated in propylene oxide, followed by embedding in a graded series of (10-100%) Spurr's epoxy resin. In the case of overnight steps, tissue was placed on the rotator at room temperature for the first and last hours, and in the refrigerator (not rotating) for the time in between. A sample protocol of the transmission electron microscope fixation procedure for spermatangia is provided in Appendix 3.

Gold-purple to silver sections (170-75 μ m thick) of embedded material were cut on a Reichert OM U3 ultramicrotome using a Dupont diamond knife. Grids were stained in saturated uranyl acetate in 70% methanol for 45 min, and then with Reynolds' lead citrate (Reynolds, 1963) for 5.5 min. Sections were viewed and photographed in Zeiss EM10A and EM10C transmission electron microscopes.

C. SPERMATIOGENESIS

Spermatangial development is the same in all *Gelidium* species in this study. Spermatangia are borne in a superficial layer (Fig. 189) on short, ultimate branchlets of gametophytes. In monoecious *G. vagum*, they occur proximal to the female area of branchlets, a short distance behind the apex. Spermatangia are initiated and mature at the same time as the more distal carpogonia. In dioecious *G. coulteri* and *G. purpurascens*, spermatangia extend practically to the apex of the branchlet, and the size of the fertile area expands as the branchlet lengthens. Spermatangia are conspicuous by being colourless and associated with a "halo" of mucilage.

Vegetative cortical cells are pigmented; their dominant cytological components are a few large chloroplasts located peripherally (Fig. 191). The nucleus is usually basal, and the central part of the cell is occupied by a vacuole (Fig. 191). In surface view, cells in male areas are paler and smaller in diameter than vegetative cortical cells, and grouped in two's or four's (Fig. 190). These smaller diameter cells are spermatangial mother cells produced by the anticlinal halving of a surface cortical cell and the subsequent division of these cells in half (Fig. 192). Spermatangial mother cells thus have pit-connections to a subtending cortical cell and/or to another spermatangial mother cell (Fig. 192).

Elongated spermatangial mother cells contain apical plastids ranging from proplastids to fully developed chloroplasts, typical of red algae, with an inner encircling thylakoid and aligned single thylakoids (Figs. 193, 203) bearing phycobilisomes. Starch grains accumulate in the basal half of spermatangial mother cells (Figs. 192, 193), and the nucleus also is basal, elongating into a central position (Fig. 193) prior to nuclear division (Fig. 194). Although a large number of cells were observed, and plants were fixed at times when, according to previous reports, the possibility of seeing dividing cells was highest (Scott *et al.*, 1980; Scott, pers. comm.; Davis and Scott, 1986; van der Meer, pers. comm.), mitotic stages were not seen. After karyokinesis, the spermatangial mother cell nucleus returns to a basal position (Fig. 194). Cytokinesis occurs in a transverse plane characteristic of the Gelidiales, dividing the spermatangial mother cell approximately in half (Fig. 204) and producing a distal spermatangium. In longitudinal sections of fertile male branches, the transverse divisions of spermatangial mother cells help distinguish spermatangial from

vegetative areas where cortical cells divide somewhat obliquely.

The spermatangium contains a prominent nucleus with condensed chromatin (Figs. 197, 201), endoplasmic reticulum and numerous mitochondria sometimes associated with dictyosomes (Figs. 195), but lacks chloroplasts and proplastids. If all chloroplasts are not successfully excluded prior to cytokinesis of the spermatangial mother cell, they must degenerate very soon after the spermatangium is cut off as extrusion of membranes is sometimes observed in the young spermatangium (Fig. 198). In some views, spermatangial mother cells appear to be expelling an entire chloroplast by either a furrow or by reforming the plasma membrane proximal to the chloroplast (Fig. 193). Soon after the spermatangium is cut off, many cored vesicles appear in the cytoplasm. Their origin is unclear, although similar cored vesicles were dictyosome-derived in developing tetrasporangia (see Chapter 7). Cored vesicles fuse with the plasmalemma (Fig. 199), contributing to a finely fibrillar, mucilage-like layer surrounding the spermatangium. The spermatangium is surrounded by the spermatangial mother cell wall (Fig. 205). One or two large spermatangial vacuoles form in the basal part of the spermatangium (Fig. 199), apparently from the coalescence of uncored, dictyosome-derived vesicles (Fig. 195), vesiculated ER (Fig. 196), or both. Spermatangial vacuoles can be discerned in the light microscope as unstained areas at the base of spermatangia (Fig. 189). Spermatangial vacuoles are filled with whorls of fibrillar material (Figs. 197, 200) and enlarge to a volume approximately equal to that of the remainder of the spermatangial cytoplasm.

Release of the spermatangial vacuole along with adjacent cytoplasm (Fig. 203) is

followed by release of a single spermatium from the spermatangium. In early stages of spermatium release, the discarded pit plug (originally between the spermatangial mother cell and spermatangium) is seen between the spermatangial vacuole and spermatangial mother cell. The new spermatangial mother cell wall is thicker and interspersed with vesicles near the pit plug (Fig. 205). The spermatangial mother cell adjacent to this area of thickened wall contains additional vesicles, some of which are released to the wall. The space between the spermatangial vacuole and spermatangial mother cell often contains many vesicle remnants. (Figs. 204, 205) that could function in gamete release. The spermatium being released is squeezed through a weakened and ruptured area of the thallus wall (Fig. 202). It is conical in shape (Fig. 206), rounding up when free of the gametophyte. The spermatium is wall-less and contains a prominent nucleus with much condensed chromatin (Fig. 206), several large, oval mitochondria and abundant cored vesicles that continue to be released to the cell exterior (Fig. 206). Chloroplasts or proplastids are absent.

Percurrent production of spermatangia occurs when the spermatangial mother cell expands into the space vacated by the previous spermatangium and cuts off another spermatangium (Figs. 205, 207). Occasionally, two spermatia are seen near the spermatangial mother cell that produced them (Fig. 205), suggesting rapid spermatium production. Loose pit plugs and successive old spermatangial mother cell wall layers indicate that at least four to five spermatangia can be produced percurrently from a single spermatangial mother cell (Figs. 207, 208).

D. COMPARISON OF SPERMATIOGENESIS IN GELIDIUM AND OTHER FLORIDEOPHYCEAN GENERA

Detailed ultrastructural studies of florideophyte spermatogenesis have been made on *Griffithsia flosculosa* (Peyrière, 1971), *Levringiella gardneri*, *Erythrocytis saccata* (Kugrens and West, 1972b), *Ptilota densa* (Scott and Dixon, 1973a), *Janczewskia gardneri* (Kugrens, 1974), *Polysiphonia harveyi*, *P. denudata* (Scott *et al.*, 1980), *P. hendryi* (Kugrens, 1980) and *Bonnemaisonia hamifera* (Simon-Bichard-Brèaud, 1971, 1972a, 1972b) and the general pattern of male gamete development is similar in all, although there are some differences in certain details. For clarification, it should be noted that Dixon (1959) used a terminology that is contrary to other authors. His "spermatangial mother cell", which expands giving rise to "spermatangia" by oblique divisions, is equivalent to a cortical cell producing two to four spermatangial mother cells (Dixon, 1959). Likewise, his "spermatangium", which divides transversely, is equal to the the spermatangial mother cell dividing to produce spermatangia (Dixon, 1959).

Kugrens' (1980) interpretation of spermatangium and spermatium in *Polysiphonia hendryi* generated terminology that also is at variance with that of other authors. He interpreted the spermatangial mother cell as producing spermatia, the wall matrix surrounding the spermatia representing the spermatangium. It is unclear whether the spermatangium also surrounds the "spermatial" mother cell, but if so, this spermatangium cannot be homologous to the spermatangium of other genera which excludes the spermatangial mother cell. Clearly the outer wall matrix, surrounding the spermatangial mother cell and developing male gametes,

was produced by the periaxial cell before production of spermatangial mother cells and is equivalent to the wall produced by other vegetative periaxial cells. Thus I have retained the usage of other authors for (e.g. Scott and Dixon, 1973a) for "spermatangial mother cell" which produces a "spermatangium" and releases a "spermatium". I do agree with Kugrens' (1980) designation spermatangial "vacuole", as opposed to "vesicle" and have followed his interpretation.

Spermatangial mother cells differ from vegetative cortical cells in that their chloroplasts are less well developed (Kugrens, 1974; Peyrière, 1974), or, if well developed (i.e. with many single parallel thylakoids), are fewer and less dominant components of the cell (Kugrens and West, 1972b), resulting in the reduced pigmentation seen at the light level. The latter is true for the *Gelidium* species studied here. Spermatangial mother cells also are less vacuolate than cortical cells (Peyrière, 1971; Scott and Dixon, 1973a; Kugrens, 1974; this study), with smaller and more basal vacuoles, except in *Polysiphonia hendryi* where spermatangial mother cells have differentiated directly from pericentral cells and are thus vacuolate (Kugrens, 1980). The transverse division of the spermatangial mother cell to produce a spermatangium is characteristic of Gelidiales and of the genera *Gracilaria* and *Hypnea* (Tazawa, 1975). Spermatangia are cut off by oblique divisions in other genera (Tazawa, 1975).

In *Gelidium* each spermatangial mother cell produces a single spermatangium at any one time, and over time sequential production of spermatangia occurs. In some species, however, (e.g. *Levringiella gardneri* and *Erythrocytis saccata*) several spermatangia are produced simultaneously by a single spermatangial mother cell

(Kugrens and West, 1972b), and percurrent production is not mentioned or visible in illustrations. In other species (e.g. *Ptilota densa*, *Janczewskia gardneri* and *Polysiphonia hendryi*) multiple, percurrent spermatangia are cut off from a spermatangial mother cell (Scott and Dixon, 1973a; Kugrens, 1974, 1980 respectively).

Chloroplasts in varying forms may be present within spermatangia or are absent (e.g. *Gelidium*). If present, they most often are rare (*Ptilota densa*: Scott and Dixon, 1973a), degenerating (*Levringiella gardneri*: Kugrens and West, 1972b), proplastids (*Erythrocytis saccata*: Kugrens and West, 1972b; *Polysiphonia hendryi*: Kugrens, 1980), or have fewer single thylakoids than vegetative cells (*Griffithsia flosculosa*: Peyrière, 1971). Organelles such as vacuoles, starch grains and the spermatangial mother cell nucleus in *Griffithsia flosculosa* (Peyrière, 1971) are positioned basally in the dividing spermatangial mother cell and are readily excluded from the spermatangium. But in *Gelidium*, well-developed chloroplasts occur apically in spermatangial mother cells. These chloroplasts appear to be released prior to spermatangial formation and then degenerate outside the cell. Evidence of expelled membranous material was seen in *Gelidium* and probably also occurs in *Levringiella gardneri*, *Erythrocytis saccata* (Kugrens and West, 1972b) and *Ptilota densa* (Scott and Dixon, 1973a).

Spermatangia show abundant signs of metabolic activity. In *Gelidium* (this study), *Griffithsia* (Peyrière, 1971, 1974) and *Bonnemaïsonia* (Simon-Bichard-Bréaud, 1972a, 1972b), mitochondria are closely associated with dictyosomes. Cored vesicles were more abundant in *Gelidium* compared to *Ptilota* (Scott and Dixon,

1973a) and *Janczewskia* (Kugrens, 1974) and are probably dictyosome-derived. There is evidence that their contents are secreted extracellularly, contributing to wall development. Although in *Griffithsia* (Peyrière, 1971, 1974), *Bonnemaisonia* (Simon-Bichard-Bréaud, 1972a), and *Janczewskia* (Kugrens, 1974), small amounts of cytoplasmic starch are present early in spermatangial development, none was seen in spermatangia of *Gelidium*. Because starch is always basal in spermatangial mother cells, it is unlikely to be included in newly formed spermatangia. Since plastids are absent, starch could not be formed *de novo* in spermatangia.

The layers surrounding spermatangia in *Gelidium* differ from the three layered wall described in *Janczewskia* (Kugrens, 1974), *Erythrocytis* and *Levringiella* (Kugrens and West, 1972b). The outermost and thickest layer in *Gelidium* consists of very loose, fibrillar, mucilage-like material (similar in appearance to the contents of the spermatangial vacuole). Late in spermatangial development a thin, granular, inner wall layer appears. From the description given by Kugrens (1974), the second layer in *Janczewskia* spermatangia may correspond to the thick mucilage-like layer of *Gelidium*. The spermatangial wall of *Polysiphonia hendryi* (Kugrens, 1980) does not appear to have any layers analogous to those in *Gelidium*. In *P. hendryi*, a dark line of compressed fibrils forms a "separating layer" at the beginning of spermatium enlargement (Kugrens, 1980, Figs. 7, 12). In Kugrens' terminology, a spermatial wall (equivalent to spermatangial wall) with a distinctive reticulate fibrillar construction forms around the spermatangium and is gelatinous in nature (Kugrens, 1980, Fig. 12). Both a separating layer and a reticulate spermatangial wall are absent in *Gelidium*, but the reticulate

wall occupies the same position as the mucilage layer in *Gelidium*. A histochemical comparison of the reticulate wall and mucilage layer would be valuable.

The spermatangial nucleus in *Gelidium* contains large amounts of condensed chromatin, but the components of the mitotic apparatus were not seen. It was proposed for *Griffithsia flosculosa* and *Ptilota densa* in which condensed chromatin also occurs that the spermatangial nucleus is arrested at prophase (Peyrière, 1974; Scott and Dixon, 1973a), hence the condensed chromatin. Contrarily, Kugrens (1974, 1980) noted that in released spermatia of *Janczewskia* and *Polysiphonia hendryi* the chromatin was dispersed.

Other electron microscope studies of spermatogenesis in florideophytes have reported a predominance of dictyosomes or ER, or a temporally changing dominance of both in spermatangia, the amount and kind of activity varying with species (Peyrière, 1974). Dictyosomes and ER vesicles are the source of secreted mucilages, wall material and the spermatangial vacuole. In *Gelidium*, spermatangial vacuoles were large, indicating much secretion. The source of this large volume of material in *Gelidium* is not obvious. Dictyosomes occasionally are seen but are not abundant, although cored vesicles believed to be derived from dictyosomes are abundant, suggesting rapid production of vesicles from few dictyosomes. Occasionally in the early stages of spermatangial vacuole formation, vesiculated ER is abundant basally, seemingly involved in formation of the spermatangial vacuole. However, ER is not apparent in spermatangia already possessing small spermatangial vacuoles.

Spermatangial vacuoles are the most prominent developmental feature of spermatangia. They are usually one to three in number and at maturity occupy up to one half the spermatangial volume. Their origin is debated; an endoplasmic reticulum (ER) origin is suggested in *Levringiella* (Kugrens and West, 1972b), *Janczewskia* (Kugrens, 1974) and *Polysiphonia hendryi* (Kugrens, 1980), whereas in *Erythrocytis* (Kugrens and West, 1972b) and *Bonnemaisonia* (Simon-Bichard-Bréaud, 1972a) the evidence indicates a dictyosome origin. In *Gelidium*, as in *Ptilota* (Scott and Dixon, 1973a), both dictyosome-derived vesicles and ER contribute to spermatangial vacuole formation. As determined by histochemical tests, the spermatangial vacuoles in *Bonnemaisonia* (Simon-Bichard-Bréaud, 1972a) and *Polysiphonia hendryi* (Kugrens, 1980) contain polysaccharides. In *Gelidium* the internal structure consists of whorls of fibrils, but in *Ptilota* it also appears as bands of granular material (Scott and Dixon, 1973a). The spermatangial vacuolar contents of *Polysiphonia hendryi* initially are granular and later develop into fibrillar material (Kugrens, 1980), and a change in chemical reactivity or change in internal appearance occurs at maturity in *Bonnemaisonia* (Simon-Bichard-Bréaud, 1972a). The position of spermatangial vacuoles also varies between taxa. In *Bonnemaisonia* two spermatangial vacuoles are produced, one of which is basal and the other apical (Simon-Bichard-Bréaud, 1971, 1972a, 1972b). They mature and are released in these positions without migration. To date, *Bonnemaisonia* is the only species studied where all spermatangial vacuoles are not basal.

The role of the spermatangial vacuole in the spermatangium has been debated. In *Gelidium* the spermatangial vacuole appears to function in release of the

spermatium. The vacuole always is released from the spermatangium before the gamete is released, and it remains approximately the same size and configuration after release. Thus it does not seem to contribute to the mucilage or adhesive coating of the spermatium, as has been suggested for *Griffithsia* (Peyrière, 1971), *Levringiella* and *Erythrocytis* (Kugrens and West, 1972b) and *Ptilota* (Scott and Dixon, 1973a). The spermatium mucilage probably is produced primarily from cored vesicles. The fact that the spermatangial vacuole never is retained within the *Gelidium* spermatium also suggests that it functions in spermatium release. In *Levringiella* (Kugrens and West, 1972b) and *Polysiphonia hendryi* (Kugrens, 1980), spermatia commonly retain their spermatangial vacuoles. No other workers have reported retention of spermatangial vacuoles in spermatia. It could reflect a real generic difference between taxa, or it could be due to accidental release of immature spermatia during electron microscopy preparation. Kugrens and West (1972b) proposed that osmotic pressure generated by the polysaccharides contained in the spermatangial vacuole forced the pit plug between the spermatangial mother cell and spermatangium to break, enabling spermatial release. However, in *Polysiphonia hendryi* (Kugrens, 1980) the pit plug ruptures by spermatangial wall deposition and spermatangium enlargement and the separating layer breaks, facilitating release of the spermatium. In several cases the pit plug in *Gelidium* was unattached on both faces, and the spermatangial mother cell showed a plasma membrane profile suggesting secretion of wall material under the plug that effectively breaks any solid connection between the spermatangium and the spermatangial mother cell. Although spermatium release could not occur with an intact pit-connection, the breaking of the pit-connection may be necessary, but not sufficient, for spermatium release. It may be difficult to separate the

consequences of pit-connection breakage and spermatangial vacuole release, as expulsion of the spermatangial vacuole and adjacent cytoplasm effectively breaks the pit-connection. A detailed ultrastructural study of mature but unreleased spermatia [sometimes seen as a second spermatium in series over a spermatangial mother cell (Fig. 205)] may resolve this question.

Dixon (1959) suggested that the lower cell of his "spermatangium" in *Gelidium*, i.e. the spermatangial mother cell, also released a spermatangium. Electron microscopy clearly shows that this does not occur, at least in the *Gelidium* species studied here.

In the Gelidiales there are morphological variations in male gametophytes or the manner in which the spermatangia are borne on gametophytes. With the exception of *Acanthopeltis japonica* (Kaneko, 1968), *Gelidium johnstonii* and *G. vagum*, all gelidioids where males are known are dioecious. Spermatangia in these plants form close behind the apex of fertile branchlets, and as the apex grows the male area lengthens. In monoecious plants, however, spermatangia are prevented from forming immediately behind the fertile branchlet apex, as this area differentiates carpogonia and nutritive filaments. In local *G. purpurascens*, male gametophytes are isomorphic with female gametophytes and tetrasporophytes and are not smaller than female gametophytes as reported for *G. latifolium* (Dixon, 1959).

Male plants or male segments of monoecious gametophytes are paler in colour than vegetative regions (Fan, 1961; Akatsuka, 1970, 1973, 1979). Electron

microscopy has shown that spermatangia lack chloroplasts and thus should be colourless. Spermatangial mother cells contain fewer and less prominent chloroplasts than vegetative cortical cells and might be expected to be somewhat pigmented. Male areas also may be recognized at low magnification by the thickened mucilage layer over spermatangia (Dixon, 1959; Akatsuka, 1970), or by the dilated and flatter apices of fertile branches (Tazawa, 1975). The latter character applies only to certain species (*Gelidium amansii*, *G. pacificum*, *Pterocladia nana*, Tazawa, 1975; *P. caloglossoides*, this work) and was not evident in *G. coulteri*, *G. johnstonii*, *G. purpurascens* or *G. vagum*. In transverse or longitudinal sections, spermatangia are distinguished easily from vegetative cortical cells by the transverse walls between spermatangial mother cells and spermatangia, and the narrower width of spermatangial mother cells. In contrast, vegetative cortical cells have oblique division planes.

Despite the differences in how and where spermatangia are borne on gametophytes, the pattern of their development in florideophytes including *Gelidium* is remarkably uniform. In addition, two species of *Gelidium* studied here could not be differentiated at the ultrastructural level.

Too few ultrastructural studies of spermatogenesis have been carried out to know yet if there are variations on the basic pattern that may be of taxonomic significance. The mechanism of exclusion or elimination of chloroplasts from spermatangia, the mode of formation of spermatangial vacuoles, the number and position of spermatangial vacuoles and the presence or absence of percurrent production of spermatangia vary in studies to date and may have some potential

for use in taxonomic studies. The mechanism of spermatium release also needs clarification.

CHAPTER VII. ULTRASTRUCTURE OF TETRASPOROGENESIS IN *GELIDIUM*

A. INTRODUCTION

Tetrasporogenesis in red algae has been studied more extensively than spermatogenesis at both light microscopy and ultrastructural levels. The origin of tetrasporangia and production of tetraspores have been noted in many gelidiaceous species. Fan (1961) remarked on the uniformity of tetrasporangial development in Gelidiales. Tetrasporangial characters (excluding arrangement on fertile branches) have not been used as taxonomic characters in Gelidiales, with the exception of the production of bispores in *Suhria vittata* and *Onikusa pristoides* (as *Gelidium pristoides*) (Fan, 1961). Tetrasporangial initials are borne laterally on subcortical cells, or "terminal in position on a lateral filament" as often stated (e.g. Fan, 1961). The young sporangium has a single pit-connection that is initially basal and becomes lower lateral as the sporangium expands. The tetrasporangium is deeply pigmented and divides into four cruciate or irregular cruciate, occasionally tetrahedral (Dixon, 1959) tetraspores. Release occurs through the degraded apical sporangial wall (Dixon, 1959). Additional sporangia are produced by the expansion of other initials after release of earlier formed sporangia.

Ultrastructurally, tetrasporogenesis has been studied in the following Ceramiales, *Griffithsia flosculosa* (Peyrière, 1969, 1970), *Levringiella gardneri* (Kugrens and West, 1972c), *Ceramium* sp. (Chamberlain and Evans, 1973), *Ptilota hypnoides* (Scott and Dixon, 1973b), *Callithamnion roseum* (Konrad Hawkins, 1974b),

Polysiphonia denudata (Scott and Thomas, 1975; Alley and Scott, 1977), *Erythrocytis montagnei* (Santisi and De Masi, 1981), two members of Corallinales, *Corallina officinalis* (Peel *et al.*, 1973), *Halptilon cuvieri* (Vesk and Borowitzka, 1984), two species of Hildenbrandiales, *Hildenbrandia rubra* and *H. occidentalis* (Pueschel, 1982), one species of Rhodymeniales, *Gastroclonium clavatum* (Gori, 1982), and one of the Palmariales, *Palmaria palmata* (Pueschel, 1979). This ultrastructural study of tetrasporangial development in *Gelidium*, was undertaken in order to determine their patterns and compare them with previous studies of other red algae.

B. METHODS

Fertile tetrasporophytes of *Gelidium coulteri*, *G. purpurascens* and *G. vagum* were collected from the field or obtained from culture isolates originating from the study area. Tissue was prepared for electron microscopy as outlined in Chapter 6. However, fixation and osmication times were longer or at a warmer temperature to allow penetration of glutaraldehyde and osmium through the thick tetrasporangial walls. Material was fixed in glutaraldehyde for 4 h. at room temperature and postfixed in osmium for 18-19 h. at room temperature. The tissue preparation protocol that was used is given in Appendix 3.

C. ULTRASTRUCTURAL OBSERVATIONS

The process of tetraspore production and maturation in *Gelidium coulteri*, *G. purpurascens* and *G. vagum* is similar at both light and electron microscope

levels, although there are differences between these species regarding the position of sporangia on stichidia. The account given here is applicable to all three local *Gelidium* species. Structures that exhibited the greatest amount of change from their appearance in vegetative cells were the nucleus, dictyosomes and wall.

1. Pre-meiotic Tetrasporangium

The presence or absence of three distinctive wall layers serves as a reliable marker for events during development of mature tetraspores from the tetrasporangial initial cell. Vegetative cortical cells (similar to Fig. 191) from which tetrasporangia differentiate, have a central vacuole, basal nucleus, starch grains, and chloroplasts that are few in number, large and peripheral. Young sporangial initials contain the remnants of a vacuole (Fig. 210), and the vacuolar contents appear to be incorporated into vesicles and secreted extracellularly (Fig. 212). Occasionally, whorls of membranes are extruded from the cytoplasm (Fig. 211). In tetrasporangial initials, chloroplasts divide producing many smaller, ovate chloroplasts. Chloroplasts are unaligned and generally peripheral, but also may be scattered throughout the cytoplasm (Fig. 214). In very young tetrasporangia, dictyosomes are absent, or are so few that they were not seen in sections of cells, and starch grains are absent. The plasma membrane of young tetrasporangia is convoluted (Figs. 210, 216). Vegetative cells have a loose, fibrillar, electron transparent wall (layer 1) (Fig. 212) that is thickened to 100-250 nm wide during differentiation and early development of the sporangium (Fig. 215). Dictyosomes and vesicles that might be contributing wall material (Fig. 216) are not abundant, thus the source of wall components is unclear.

In young tetrasporangia the interphase nucleus is enlarged and central (Figs. 214, 215) and contains a large nucleolus with an uneven granular composition (Figs. 213, 219). Nucleolar vacuoles are evident as areas of the nucleolus with the same electron density as the nucleoplasm (large arrows, Fig. 213), and have been associated with RNA synthesis (Peel *et al.*, 1973). Pores are abundant in the nuclear envelope (arrowheads, Fig. 213) and perinuclear endoplasmic reticulum (PER) is present (small arrows, Fig. 213, Fig. 218, arrows, Fig. 219). Mitochondria are concentrated in the immediate vicinity of the nucleus. Dictyosomes appear just prior to karyokinesis and are small, flattened and up to 12 cisternae thick, with ends of distal cisternae inflated and saccate (Fig. 221). Larger cisternae contain an elongate core of moderately electron dense, fibrillar material (Fig. 221). The cisternal periphery, as well as the entire contents of young cisternae, are electron transparent and sparsely fibrillar. There is a close association between mitochondria and the forming face of dictyosomes (Fig. 221). The narrow space between these organelles is unlike the surrounding cytoplasm in that it is free of ribosomes. Small, narrow, lenticular starch grains also appear prior to karyokinesis (Figs. 214, 215, 218, 219), first located centrally in the vicinity of the nucleus and between chloroplasts, and later scattered throughout the tetrasporangium.

Actual meiotic division stages or synaptonemal complexes were not observed in these preparations. Several elongated and spindle shaped nuclei with depressions at their ends (Figs. 218, 219) and one with a narrow waist and chromatin (Fig. 217) probably were preparing for division. Somewhat older tetrasporangia with two nuclei but had not yet undergone cytokinesis (Fig. 220).

2. Post-meiotic Tetrasporangium

Following karyokinesis, as the tetrasporangium enlarges, starch grains, chloroplasts and dictyosomes become more abundant (Fig. 220). Starch grains change from lenticular to ovate in shape (Figs. 220, 224). Chloroplasts divide by pinching into two and become somewhat more elongate. They are well developed, with an encircling thylakoid interior to the double chloroplast membrane, many parallel, single thylakoids, plastoglobuli and areas of DNA (Figs. 221, 225). Dictyosomes become more prominent, particularly around the periphery of the sporangium. At low magnification they are noticable as clusters of sacs (Fig. 223) that still are flat but composed of more cisternae with larger inflated sacs. They are abundant in the tetrasporangium at all stages of development. Mitochondria are small relative to chloroplasts and are closely associated with the forming face of all dictyosomes (Fig. 221). Endoplasmic reticulum (ER) also is present in tetrasporangia, although often difficult to distinguish in the dense cytoplasm (Figs. 221, 222).

A second tetrasporangial wall layer (layer 2) (Fig. 222) is deposited inside the first layer. This occurs when starch grains and chloroplasts have become abundant in the cytoplasm (Figs. 220, 224) during karyokinesis but before cytokinesis. Layer 2 (arrow, Fig. 227) is narrower (20-25 nm wide) and more densely fibrillar than layer 1 and of medium electron density, appearing as a grey band between white (layer 1) and black (layer 3) bands (Figs. 227, 232). It is deposited by abundant dictyosome- (Fig. 225) and ER-derived vesicles (Figs. 222, 225).

3. Tetrasporangium at Cytokinesis

The tetrasporangium undergoes cytokinesis at the two-nucleate stage, observations supported by light microscope studies on hematoxylin-stained material. The second meiotic division then occurs in both cells followed by another cytokinesis in perpendicular planes, producing a cruciately divided tetrasporangium with four uninucleate tetraspores (Fig. 78). Each tetraspore nucleus is small and inconspicuous, obscured by the great abundance of other organelles and inclusions. Chloroplasts and starch grains are very abundant (Fig. 226). In some mature tetraspores, chloroplasts are oriented radially around the nucleus (Fig. 229), but this arrangement does not seem to be universal. Starch grains have reached their maximum size in the tetrasporangium after cytokinesis (tetraspores) (Fig. 226). They are electron transparent, non-membrane bound and appear to lack substructure although dark, shadow-like marks make them appear three dimensional and angular (Fig. 228). Endoplasmic reticulum occurs in the peripheral cytoplasm of older tetrasporangia contributing vesicles to wall formation (Fig. 231).

In tetrasporangia that are undergoing the second cytokinesis, and shortly thereafter, dictyosomes are abundant throughout the cytoplasm (Fig. 226), but not as prominent as in earlier stages. They are small and obscured by an abundance of chloroplasts and starch grains. Dictyosomes also have undergone a striking morphological change. They are distinctly concave; all of the approximately 9-12 cisternae per stack are strongly curved (Figs. 228, 230). There is a line of electron dense material inside the closely associated cisternae of the younger

(forming face) half of the dictyosome (arrowhead, Fig. 230) that is progressively more restricted in extent in the central region of more mature cisternae (Fig. 230). The dark material occurs in closely appressed, narrowest parts of cisternae and is absent from the saccate ends. Cisternae that are innermost in the cup that is formed are oldest. Their inflated sacs separate and are released as spherical vesicles (cored vesicles) which have an electron dense core and a medium density fibrous periphery (Fig. 230). Cored vesicles release their contents to the cell wall, contributing to its formation (Fig. 231). Mature tetraspores have few cored vesicles and visible dictyosomes.

The third and final wall layer of the tetrasporangium (layer 3) is produced at cytokinesis (Figs. 226, 227, 232). It is 25-55 nm wide, stains strongly with osmium and is densely fibrillar, with parallel fibrils much more clearly aligned than in other layers (Fig. 227). Soon after layer 3 is completed, a layer of whorled fibrillar material, similar to the mucilage produced by spermatangia and contained in spermatangial vacuoles, appears internal to layer 3 and surrounds the tetraspores (Fig. 232). As in spermatangia, this material represents the contents of dictyosome-derived cored vesicles (Figs. 231, 232).

D. DISCUSSION

General trends of developmental changes during tetrasporogenesis in *Gelidium* agree with patterns observed in other florideophytes. An extensive comparison and discussion of tetrasporogenesis was presented by Vesik and Borowitzka (1984). Some differences in details do occur between the species, and these may be

taxonomically important. The tetrasporangial wall differs from the wall of the vegetative cell in most species (e.g. *Ptilota hypnoides*, Scott and Dixon, 1973b; *Levringiella gardneri*, Kugrens and West, 1972c; *Haliptilon cuvieri*, Vesik and Borokitzka, 1984), and a proteinaceous (Pueschel, 1979) cuticle forms [e.g. in *Hildenbrandia rubra*, *H. occidentalis* (Pueschel, 1982), *Palmaria palmata* (Pueschel, 1979), *Callithamnion roseum* (Konrad Hawkins, 1974b) and *Ptilota hypnoides* (Scott and Dixon, 1973b)]. It has been proposed that the cuticle and tetrasporangial wall function to isolate the tetrasporangial cytoplasm from the influence of vegetative cells and sporangia at other developmental stages (Vesik and Borowitzka, 1984). In *Gelidium*, a cuticle was not apparent and neither was a unique wall formed early in tetrasporangial development; the initial wall thickens and a second wall layer is not deposited until karyokinesis.

Division figures and synaptonemal complexes were not observed in *Gelidium*. Synaptonemal complexes are definitive proof of meiosis, but have been seen in few studies (Kugrens and West, 1972a, *Janczewskia gardneri*, *Levringiella gardneri*, *Gonimophyllum skottsbergii* and *Polycoryne gardneri*; Pueschel, 1979, *Palmaria palmata*; Broadwater, Scott and Pobiner, 1986a, *Dasya baillouviana*). They occur in few sections because of their small size and thus are less likely to be seen than larger, more conspicuous indicators of nuclear division, such as spindle shaped nuclei and division furrows.

Increase in chloroplast number throughout tetrasporangial development occurs by pinching of single chloroplasts into two. This has been observed in *Gelidium* and reported in *Griffithsia flosculosa* (Peyrière, 1969), *Ptilota hypnoides* (Scott and

Dixon, 1973b), *Palmaria palmata* (Pueschel, 1979), *Gastroclonium clavatum* (Gori, 1982) and *Halptilon cuvieri* (Vesk and Borowitzka, 1984). Early in the development of *P. palmata* tetrasporangia, rapidly replicating chloroplasts have the appearance of proplastids with few or no single parallel thylakoids (Pueschel, 1979), while later in development plastids are differentiated with several parallel thylakoids. Chloroplasts in early stages of tetrasporogenesis in *H. cuvieri* had few thylakoids, but later became fully developed (Vesk and Borowitzka, 1984). Chloroplasts in *Gelidium* and *Erythrocytis montagnei* (Santisi and De Masi, 1981) are well developed at all stages of tetrasporogenesis; proplastids were not observed in this study.

The importance of dictyosomes and ER in secretory activity is recognized in all ultrastructural studies of tetrasporogenesis (e.g. Peyrière, 1970; Chamberlain and Evans, 1973; Konrad Hawkins, 1974b; Alley and Scott, 1977). During periods of wall deposition, dictyosomes and dictyosome-derived vesicles become more abundant, particularly in the peripheral cytoplasm, and cisternae increase in number, becoming more dilated and hypertrophied. After wall and mucilage secretion is reduced (in mature tetraspores), dictyosomes decrease in dilation, size and number.

Many observers of tetrasporogenesis report or illustrate close associations between mitochondria and the forming face of dictyosomes (Peyrière, 1969, 1970; Kugrens and West, 1972c; Chamberlain and Evans, 1973; Peel *et al.*, 1973; Scott and Dixon, 1973b; Konrad Hawkins, 1974b; Scott and Thomas, 1975; Alley and Scott, 1977; Pueschel, 1979, 1982; Santisi and DeMasi, 1981; Gori, 1982; Vesk

and Borowitzka, 1984; this study). Dark layers occur between adjacent, closely-appressed cisternae in the younger half of the hemispherical dictyosome. These have been likened to cementing layers (Konrad Hawkins, 1974b), but their nature is not known, and they have not been reported in other plants and animals (Alley and Scott, 1977). Products of dictyosomes also change through time, evidenced by the variety of vesicles reported to be dictyosome-derived. Without histological information it is difficult to determine the homology of vesicles reported as fibrillar (Kugrens and West, 1972c, *Levringiella gardneri*), granular (Kugrens and West, 1972c, *Levringiella gardneri*; Chamberlain and Evans, 1973, *Ceramium* sp.), dense (Peyrière, 1969, *Griffithsia flosculosa*), with a fibrillar core (Chamberlain and Evans, 1973, *Ceramium* sp.; Santisi and De Masi, 1981, *Erythrocytis montagnei*), and granular with a fibrillar outer region (Chamberlain and Evans, 1973, *Ceramium* sp.). Late in cytokinesis, dictyosomes of *Gelidium* (this study), *Levringiella gardneri* (Kugrens and West, 1972c), *Ceramium* sp. (Chamberlain and Evans, 1973), *Callithamnion roseum* (Konrad Hawkins, 1974b), *Erythrocytis montagnei* (Santisi and De Masi, 1981), *Palmaria palmata* (Pueschel, 1979), *Gastroclonium clavatum* (Gori, 1982) and *Callithamnion roseum* carpospores (Konrad Hawkins, 1974a) have a distinctive hemispherical morphology and produce cored vesicles. Peyrière (1970) reported positive tests for acid mucopolysaccharides within cored vesicles formed at two different developmental stages in *Griffithsia flosculosa*, and Tripodi and De Masi (1975) found that cored vesicles of carpospores in *Pterocladia capillacea* and *Polysiphonia sertularioides* (similar in appearance to *Gelidium* cored vesicles in tetrasporangia) gave a positive reaction to Thiery's test for polysaccharides. These cored vesicles are sometimes close to the plasma membrane, suggesting their contents are being

released to the wall (Tripodi and De Masi, 1975). The contents of cored vesicles in *Gelidium* (this study) and *Erythrocytis montagnei* (Santisi and De Masi, 1981) are secreted to the inner tetrasporangium wall or mucilage layer and are absent from released tetraspores (Santisi and De Masi, 1981). Similar appearing vesicles are believed to function in spore adhesion in other species (Vesk and Borowitzka, 1984, *Halptilon cuvieri*).

Dictyosome cisternae undergo a maturing process as they move through the stack (by addition and attrition of other cisternae) from the proximal (forming) face to the distal face. Alley and Scott (1977) proposed that they arise at the forming face from the fusion of small, ER-derived vesicles, and vesicles in transition between ER and the forming face of dictyosomes were seen in *Erythrocytis montagnei* (Santisi and De Masi, 1981). In *Gelidium*, ER occurs near the forming face, but vesicles never were seen in the space between mitochondria and the youngest cisternae. It is possible that small contributing vesicles were not sectioned or that they are added laterally and not centrally. Konrad Hawkins (1974b) stated that ER vesicles from chloroplast blebs fuse to form cisternae, and also that at least some cisternae contain chloroplast enzymes capable of starch degradation, as cisternae were seen near eroding starch grains. In *Gelidium*, chloroplasts in the general vicinity of dictyosomes showed no evidence of special positioning near dictyosomes, or of blebbing, and starch grains were not in an eroding or eroded state. Vesicle production from dictyosomes is by release of entire cisternae (Peyrière, 1969, 1970; Alley and Scott, 1977) or release of dilated ends of cisternae as vesicles (Konrad Hawkins, 1974b). In *Gelidium*, dictyosome-derived vesicles are smaller than a whole cisternum, indicating that

vesicles probably are derived from cisternal ends.

Endoplasmic reticulum also is known to produce striated vesicles (Kugrens and West, 1972c) and mucilage vesicles (Pueschel, 1979). In *Gelidium*, vesicles containing membranes or smaller vesicles, similar to multivesicular bodies or lomasomes (Scott and Dixon, 1973b; Chamberlain and Evans, 1973), release their contents to the wall.

Endoplasmic reticulum is associated with the nucleus at meiosis as PER. PER was not abundant in *Gelidium*, but is extensive, oriented parallel to the nuclear envelope or radially at different times in *Corallina officinalis* (Peep *et al.*, 1973) and *Haliptilon cuvieri* (Vesk and Borowitzka, 1984), and believed to be related to high metabolic and synthetic activity of the nucleus prior to, and throughout, meiosis. Extensive, radially-oriented PER may be characteristic of Corallinales; it has only been reported from this order, although it is absent in some corallines (e.g. *Jania rubens* (Linnaeus) Lamouroux, Peel *et al.*, 1973; *Lithothrix aspergillum* Gray and *Fosliella* sp., Vesk and Borowitzka, 1984). Endoplasmic reticulum also is important in inner tetrasporangium wall formation in *Haliptilon cuvieri* (Vesk and Borowitzka, 1984) and is present but not conspicuous at the cytoplasm periphery in *Gelidium*.

Vesicles in *Gelidium* tetrasporangia release contents to the wall by fusing with the plasma membrane. No vacuoles, similar to those reported in *Corallina officinalis* (Chamberlain and Evans, 1973), *Ptilota hypnoides* (Scott and Dixon, 1973b), *Polysiphonia denudata* (Alley and Scott, 1977), *Erythrocytis montagnei*

(Santisi and De Masi, 1981) and *Griffithsia flosculosa* (Peyrière, 1970) were seen. Mucilage sacs, present in *Palmaria palmata* (Pueschel, 1979), also were not observed in *Gelidium*.

Deposition of a series of cell wall layers results from the cytoplasmic secretory activity described above. Unlike all other species where tetrasporogenesis has been studied (Vesk and Borowitzka, 1984), *Gelidium* does not deposit a tetrasporangial initial wall early in differentiation that is different from the vegetative wall. A histochemical study of tetrasporangium wall layers in *Gelidium* would enable comparisons with vegetative walls, as the vegetative wall of *G. pacificum* Okamura has an outer sulfated polysaccharide layer and an inner cellulosic layer (Akatsuka and Iwamoto, 1979). Based on position, appearance and time of formation, the outer, thick, loosely fibrillar wall (layer 1) of the *Gelidium* tetrasporangium is probably equivalent to the tetrasporangial mother cell wall in *Levringiella gardneri* (Kugrens and West, 1972c) and *Ptilota hypnoides* (Scott and Dixon, 1973b), layer three in *Callithamnion roseum* (Konrad Hawkins, 1974b), and the inner tetrasporangial wall in *Palmaria palmata* (Pueschel, 1979), *Hildenbrandia rubra* and *H. occidentalis* (Pueschel, 1982). The medium density, grey wall (layer 2) in *Gelidium* is different from layer 1 and is similar in position and appearance to the tetrasporangial initial wall in *Ptilota hypnoides* (Scott and Dixon, 1973b). The last wall layer laid down in *Gelidium* (layer 3) is continuous with the dark layer separating tetraspores at cytokinesis, and a similar layer is recognizable in other genera. A mucilage layer also cleaves the tetrasporangium in other florideophyte genera (e.g. *Hildenbrandia*, Pueschel, 1982; *Halitilton*, Vesk and Borowitzka, 1984). This layer is recognizable by its position with respect to

the dark, inner wall and its swirled fibrillar appearance. Peyrière (1970) found in *Griffithsia flosculosa* that the last type of tetrasporangial secretion, which appears similar to the mucilage-like material in *Gelidium*, reacted positively for mucopolysaccharides in histochemical tests.

In all ceramialean and corallinean species studied ultrastructurally to date, tetraspores are tetrahedrally or zonately (respectively) arranged in the sporangium. Both meiotic divisions of the nucleus are completed before cytokinesis, and cleavage is simultaneous (Kugrens and West, 1972c, *Levringiella gardneri*; Scott and Dixon, 1973b, *Ptilota hypnoides*; Chamberlain and Evans, 1973, *Ceramium* sp.; Alley and Scott, 1977, *Polysiphonia denudata*; Vesik and Borowitzka, 1984, *Halptilon cuvieri*; Broadwater, Scott and Pobiner, 1986b, *Dasya baillouviana*) except in *Erythrocytis montagnei* where the cleavage furrow is half completed before karyokinesis is over (Santisi and De Masi, 1981). Cytokinesis is initially sequential, and later simultaneous in *Palmaria palmata* with cruciately divided tetrasporangia. (Pueschel, 1979) and in the zonately to cruciately divided tetrasporangia of *Hildenbrandia rubra* and *H. occidentalis* (Pueschel, 1982). Pueschel (1979, 1982) observed the first furrow forming after meiosis and not being completed until after the second perpendicular furrows had been initiated. *Gelidium* also has cruciately divided tetrasporangia, but cytokinesis was not observed ultrastructurally. Light microscope studies with hematoxylin-stained tetrasporangia suggested that division is completely sequential and that the second meiotic division occurs after the first cytokinesis.

In summary, whereas tetrasporogenesis follows a similar pattern in the

florideophytes studied to date, some differences between genera have been recorded such as extent and orientation of PER, types, functions and sources of vesicles, and timing of cleavage furrow development. As in spermatogenesis, there are not yet enough studies on several members of a taxon (i.e. genus, family, order) to determine whether these differences are phylogenetically significant or whether differences merely represent variations among individuals.

CHAPTER VIII. GENERAL DISCUSSION AND CONCLUSION

In spite of the great confusion confounding the taxonomy of *Gelidium* and *Pterocladia* species (e.g. Dixon, 1958), it is possible to determine species, if the range of morphological and anatomical variability within a species is studied and determined for each taxon within a restricted geographic area. In this study, conducted in British Columbia and northern Washington, species were assigned to genera on the basis of cystocarp morphology. Where cystocarps were not known for local species (i.e. *G. coulteri*, *P. caloglossoides*), ecological, vegetative and tetrasporangial characters were used to assign names to taxa. Although six taxa were recorded originally from the area, it was found in this study that only four species of Gelidiales are present: *Gelidium coulteri*, *G. purpurascens*, *G. vagum* and *P. caloglossoides*. A key was constructed (Fig. 5) to enable identification of these species. An emphasis on biogeographic and developmental patterns of reproductive structures as well as vegetative features, and on ecological characters may prove useful in further taxonomic determinations of gelidioid algae.

To date, the only character that can be used reliably to distinguish between *Gelidium* and *Pterocladia* is the number of cystocarp locules. However, some of the apical architecture characters noted by Rodriguez and Santelices (1987, unpubl. mscr.) show potential for separating some of the species of these genera vegetatively. Two of these characters, similarity/dissimilarity of apical and lateral initials and the indentation of lateral initials, were examined in the three *Gelidium*, *G. coulteri*, *G. purpurascens* and *G. vagum*, and the one *Pterocladia* species, *P. caloglossoides*, from the study area. Apical and lateral initials of the

local gelidiaceous species were found to conform to the pattern: both types of initials are similar and domed in *Pterocladia*, but apical initials are domed and lateral initials are dissimilar, inverted conical in *Gelidium*. The second character given by Rodriguez and Santelices (1987, unpubl. mscr.) was not consistent for *P. caloglossoides* although they suggested that this character might have exceptions in some *Pterocladia* species, including *P. caloglossoides*. Thus this second character of indentation of initials is incapable of differentiating between *Pterocladia* from *Gelidium* in the study area. In the present study, neither apical nor lateral initials are indented between cortical lobes in *P. caloglossoides*. Thus the similarity in shape of apical and lateral initials in *Pterocladia* species, and the dissimilarity of these initials in *Gelidium* species, in addition to number of cystocarp locules, are characters that may be used to separate *Gelidium* and *Pterocladia*.

Gelidium vagum undergoes a regular *Polysiphonia*-type life history in culture, and the common occurrence of gametophytes and tetrasporophytes suggests the same is operating in the field. According to culture evidence and field observations *G. purpurascens* in the study area is capable of, but probably rarely actually completes, a *Polysiphonia*-type life history *in situ*. Fertile male gametophytes grew from tetraspores of *G. coulteri* in culture, but there are no field collections of fertile gametophytes. The complete *Polysiphonia*-type life history probably occurs only occasionally, if at all, in *G. coulteri* from the study area and vegetative or apomictic mechanisms probably sustain the population most of the time. *Pterocladia caloglossoides* populations also persist predominantly vegetatively or apomictically. Chromosome counts of $n=14-15$ were obtained from undivided

tetrasporangia of *G. vagum*.

The pattern of spore germination was found to be similar for both carpospores and tetraspores. A single germ tube grows from the spore, the entire cytoplasmic contents of the spore evacuate into the germ tube and a wall cuts off the germ tube from the empty spore. The germ tube divides unequally to form a concave and a fusiform cell. The primary attachment rhizoid forms from a derivative of the concave cell, and the sporeling apical cell forms from a fusiform cell derivative. As apical organization is established the subapical cell produces two lateral periaxial cells.

Studies of developmental patterns in local gelidiaceous species revealed some previously unknown or unsubstantiated aspects. Hommersand and Fredericq (1988) recently proposed that carpogonia in Gelidiales are intercalary and that sessile carpogonia reported by previous workers (Dixon, 1959; Fan, 1961) are non-functional, but provided no data. The present study showed that the fusion cell is initiated from an intercalary carpogonium. In light of Hommersand and Fredericq's (1988) observations and the corroborative findings of this study, female gamete and carposporophyte development needs to be followed in detail for a wide range of other gelidiaceous species. Such an expanded study is predicted to reveal developmental variations that may be important as ordinal characters.

Ultrastructure of spermatogenesis in Gelidiales was documented for the first time in this study. Exclusion of apical chloroplasts from the spermatangial mother cell is accomplished by the previously unreported mechanism of an ingrowing furrow

that cuts off the cytoplasm containing the chloroplast. The spermatangium cut off from the spermatangial mother cell is achloroplastic. A spermatangial vacuole develops in the spermatangium from both dictyosome-derived vesicles and vesiculated endoplasmic reticulum. The spermatangial vacuole is released from the spermatangium prior to spermatium discharge. The percurrent production of at least four spermatangia from a single spermatangial mother cell occurs in *Gelidium*.

Ultrastructure of tetrasporogenesis in a member of the Gelidiales was documented here for the first time. The stage of wall development can serve as a marker for developmental events in the tetrasporangium. Prior to meiosis, the nucleus is large, dominating the cytoplasm, and the wall is thickened. Following karyokinesis, a second distinctive wall layer is deposited and chloroplasts and starch grains increase in number. Dictyosomes become more abundant and the number of cisternae increase and cisternae become more inflated. At the second cytokinesis a dark wall layer is deposited around the tetrasporangium and in the cleavage furrow. Starch grains and chloroplasts dominate the cytoplasm. Dictyosomes are strongly curved and young cisternae contain a dark-staining material where they are closely appressed. Cored vesicles are produced by the dictyosomes and subsequently release their contents forming a whorled fibrillar layer inside the dark tetrasporangium wall at the second cytokinesis.

This study is presented as a contribution to the taxonomy and biology of Gelidiales with a focus on representatives of the order in British Columbia and northern Washington. It is hoped that other workers will find these observations

useful in their studies of gelidioid algae on both a local and wider geographic scale.

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FIGURES

Abbreviations Used in Figures.

AP	apical cell
C	carposporophyte
CL	chloroplast
COR	cortex
CPG	carpogonium
CS	carposporangium
D	dictyosome
ER	endoplasmic reticulum
FC	fusion cell
G	gonimoblast
LO	locule
N	nucleus
NC	nutritive chain
NO	nucleolus
OS	ostiole
P	attachment pad
PA	periaxial cell
PC	pit connection
PL	second order plate
PR	prostrate axis
R	rhizine
SMC	spermatangial mother cell

ST	starch grain
SV	spermatangial vacuole
UP	upright axis
W	wall
1°	cell of first order filament (axial file)
2°	cell of second order filament

Fig. 1. Map of study area and area of enlargement maps in Figs. 2, 3, 4.

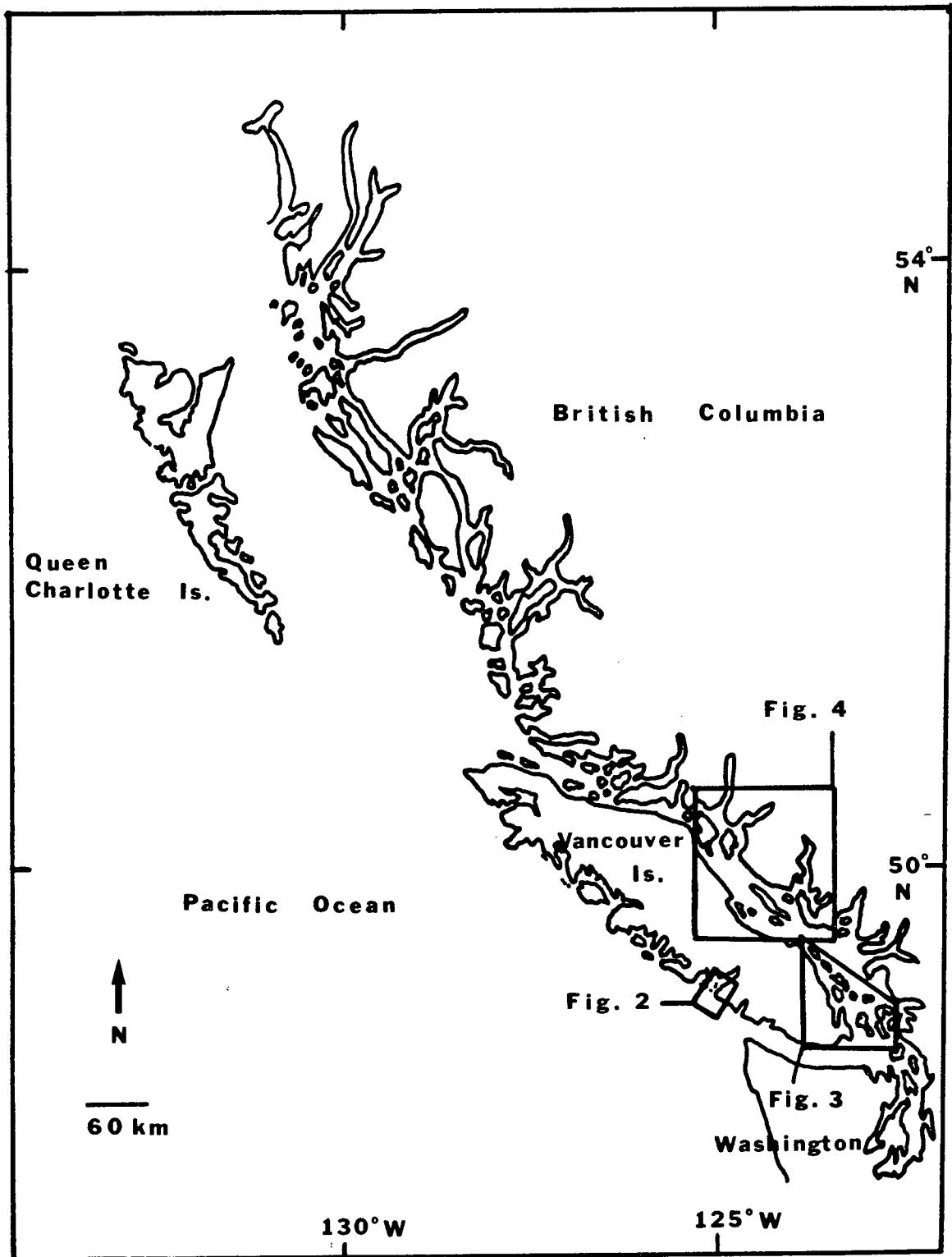


Fig. 2. Barkley Sound collection sites, numbered as follows: 1. Brady's Beach, 2. Diplock Is., 3. Dixon Is., 4. Dodger Channel, 5. Geer Islets, 6. Haines Is., 7. Kirby Pt., 8. Meade Islôts, 9. Roquefeuil Bay, 10. Ross Islets.

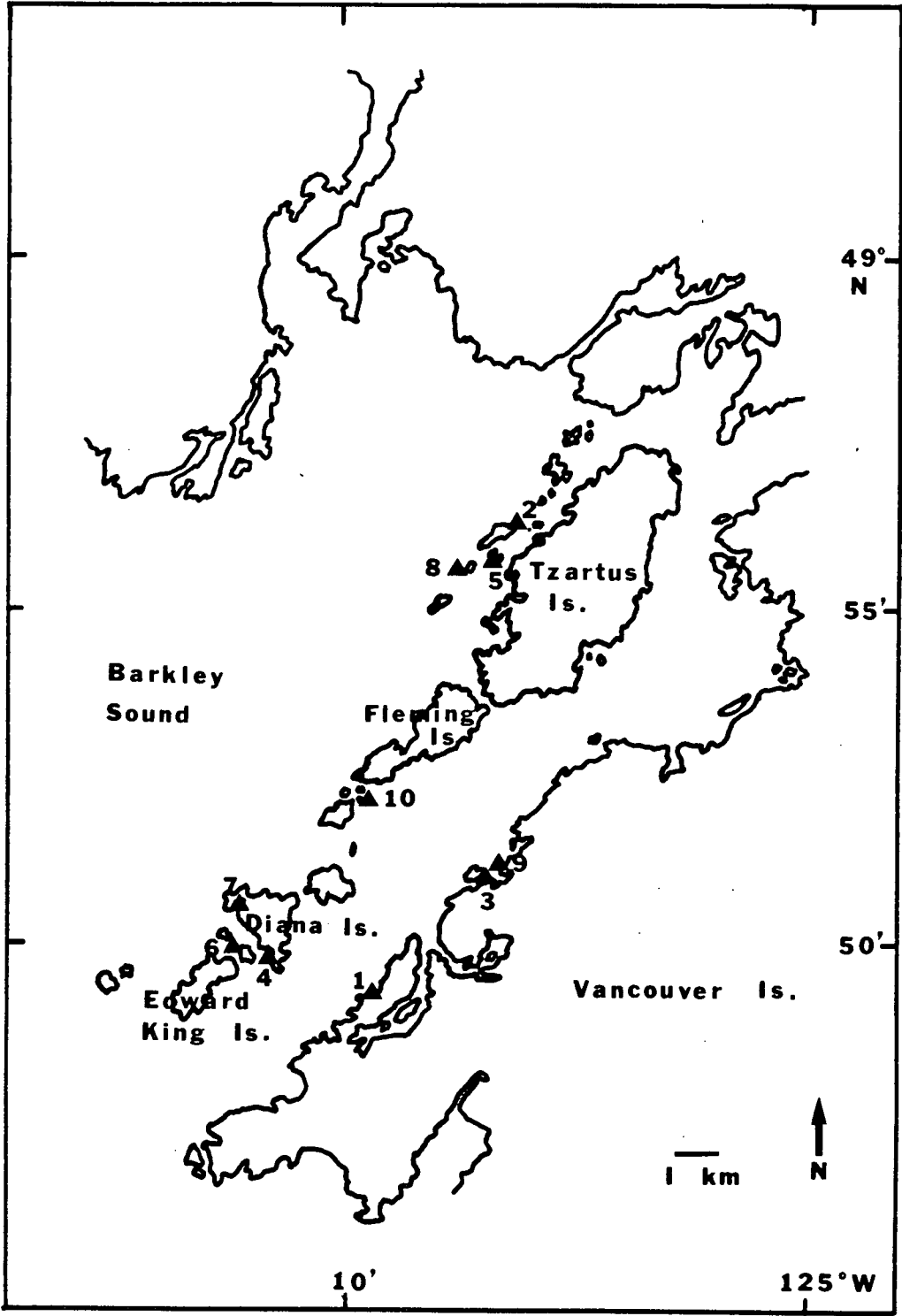


Fig. 3. Southern Vancouver Island, Gulf Islands and San Juan Islands collection sites, numbered as follows: 11. Madrona Pt., 12. Georgina Pt., 13. False Narrows, 14. Orlebar Pt., 15. Whalebone Bay.

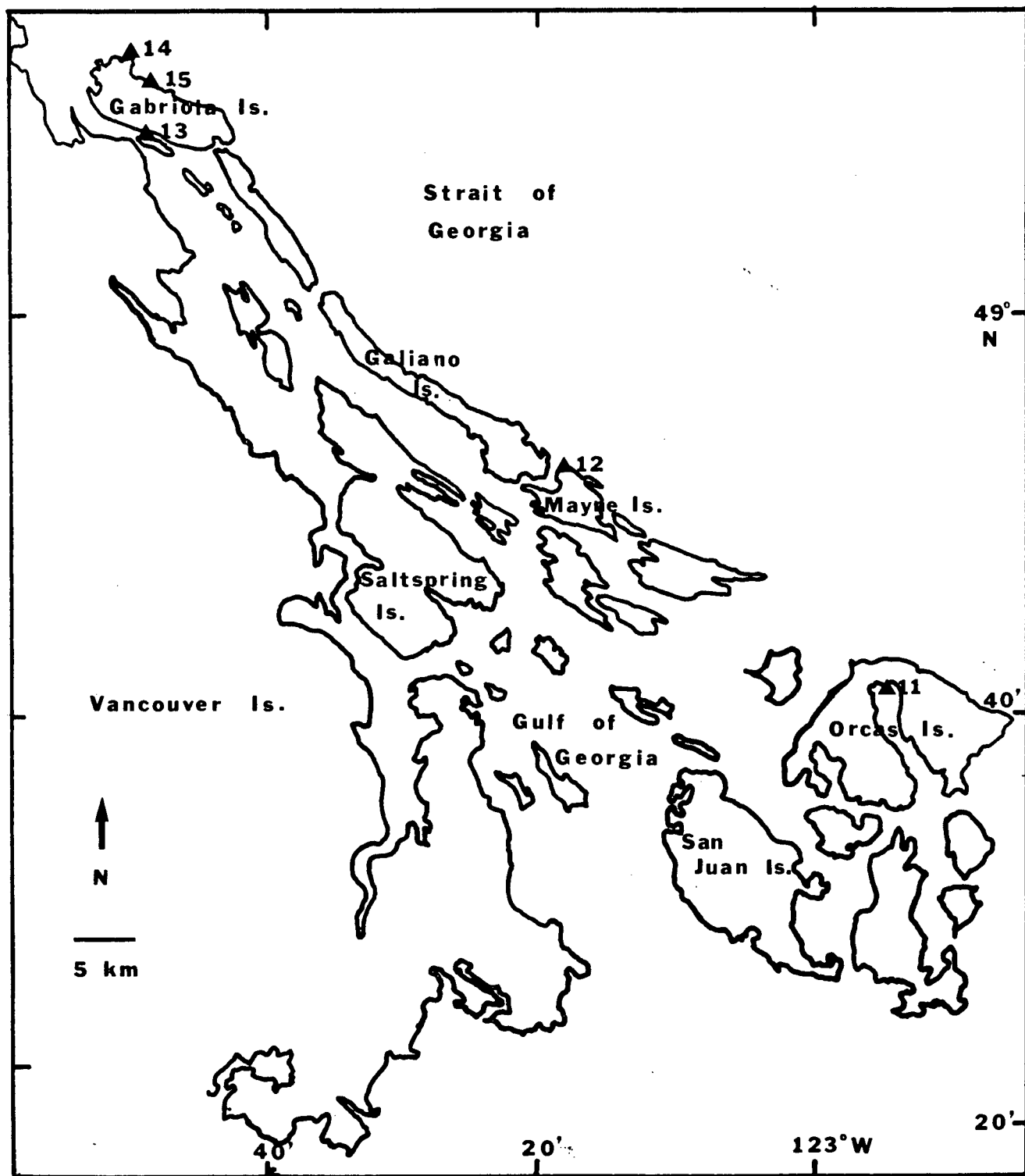


Fig. 4. Strait of Georgia collection sites, numbered as follows: 16. Earls Cove, 17. Finn Cove, 18. Frolander Bay, 19. Halfmoon Bay, 20. Denman Is., 21. Ford Cove, 22. Galleon Pt., 23. Helliwell Park, 24. Tribune Bay.

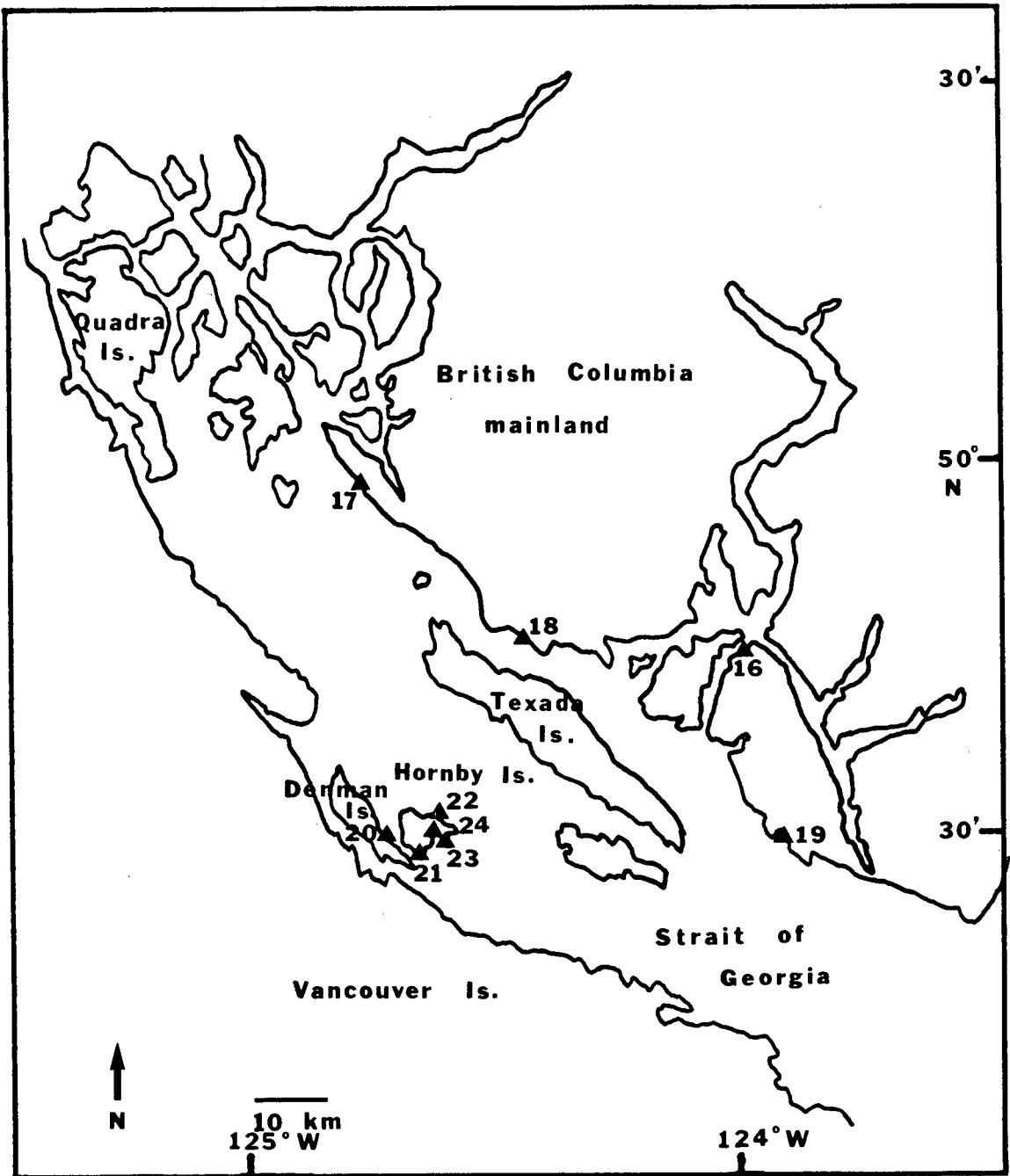
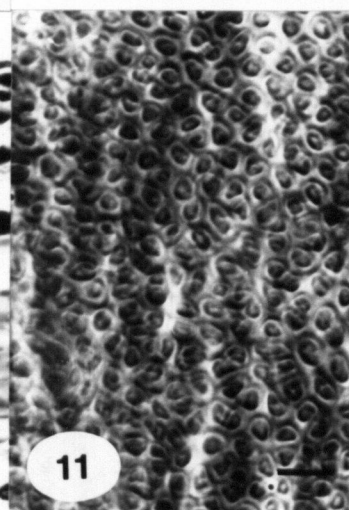
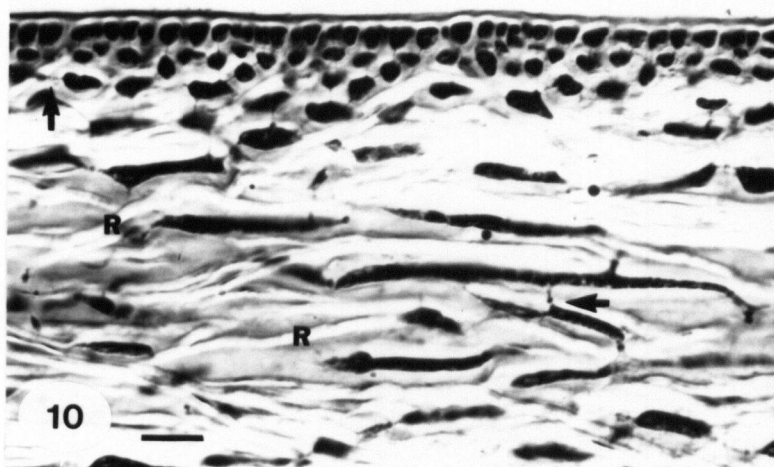
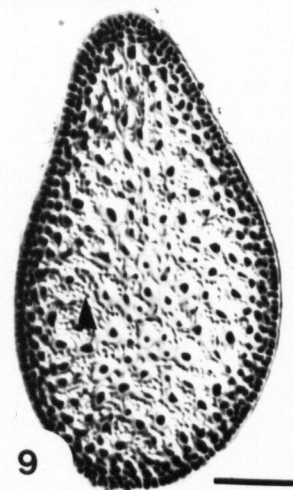
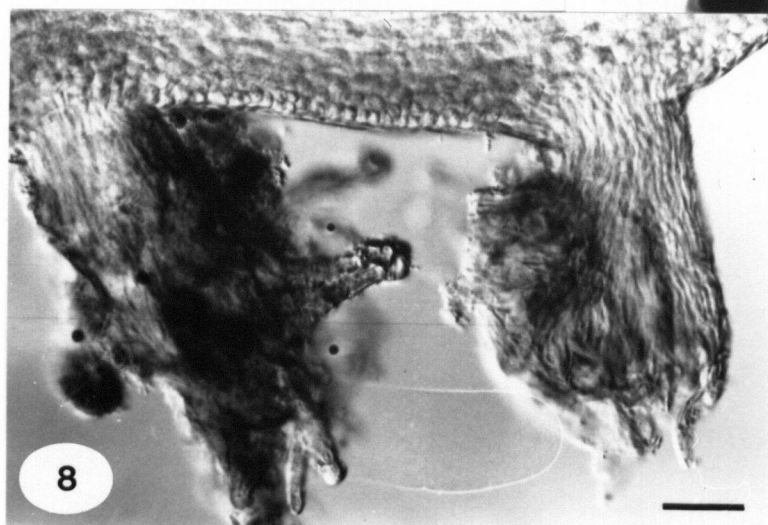
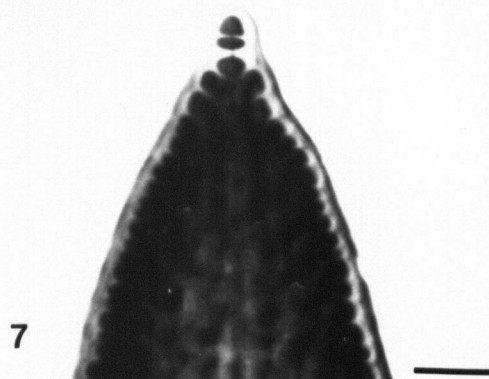
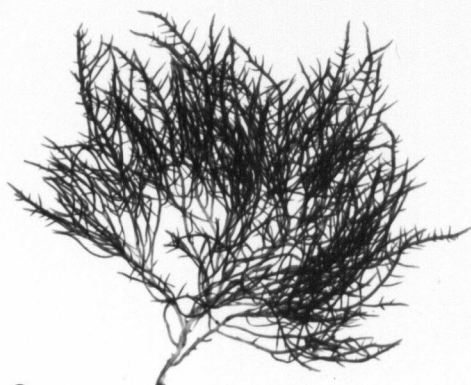


Fig. 5. Key to Gelidiales in British Columbia and northern Washington.

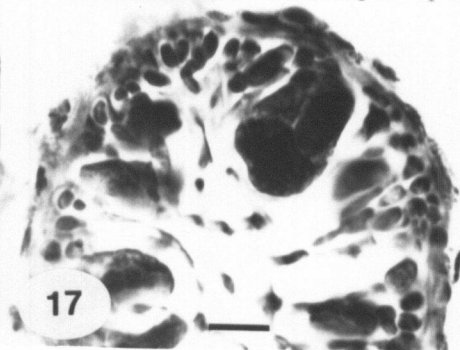
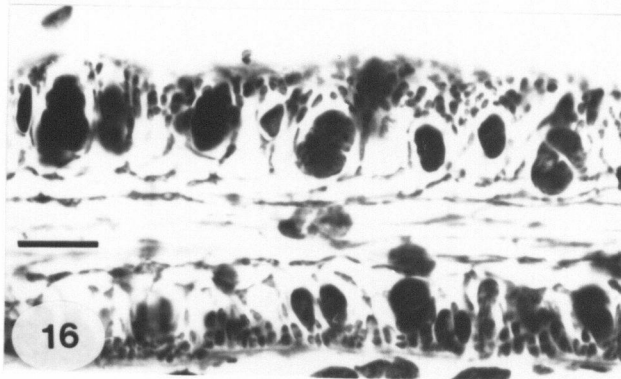
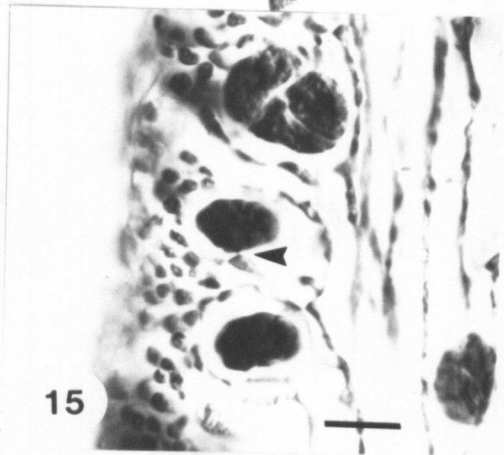
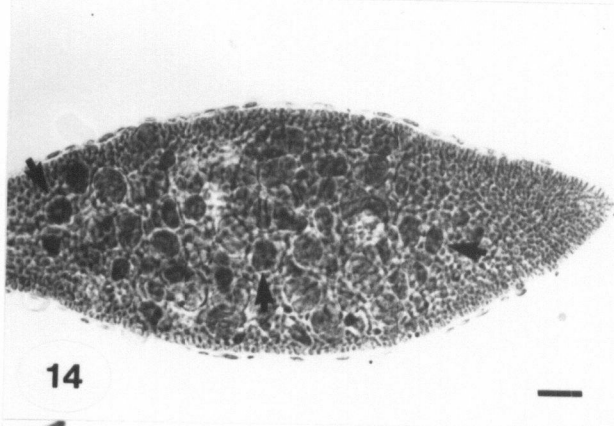
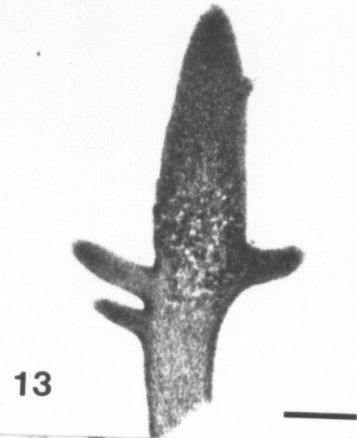
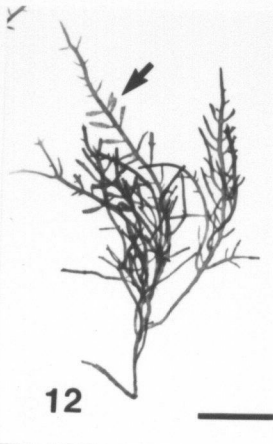
- 1a. Plant dark red; mostly of branched, flattened, creeping axes, frequently forming attachment pads at branch nodes; uprights present or absent, never more than 2 cm tall, sparse branches approximately 90° to axis; tetrasporangia in V's... *Pterocladia caloglossoides*
- 1b. Plant red to yellowish-red, dark red to maroon or brown to brownish-red; creeping axes branched, terete, with attachment pads usually not formed at branch nodes; uprights dominant, usually more than 2 cm tall, well branched, branches 30-60° to axis; tetrasporangia not in V's 2
- 2a. Plant intertidal, brown to brownish-red; forms a turf; usually two orders of branches and many short second order branches; gametophytes not known from British Columbia or northern Washington *Gelidium coulteri*

- 2b. Plant shallow subtidal (0-13 m); red to yellowish-red or dark red to maroon; does not form a turf; branching to three to four orders with abundant second, third and fourth order branches; gametophytes rare or common in British Columbia and northern Washington..... 3
- 3a. Plant robust, cartilaginous; portions of plant with pyramidal aspect; axes terete to compressed; tetrasporangial stichidia with narrow sterile margin; dioecious; third order filaments of mature cystocarp (between second order plate and pericarp) intact..... *Gelidium purpurascens*
- 3b. Plant soft and lax; not triangular in aspect; axes compressed to flattened; tetrasporangial stichidia lack sterile margin; monoecious; lacks intact third order filaments in mature cystocarp..... *Gelidium vagum*

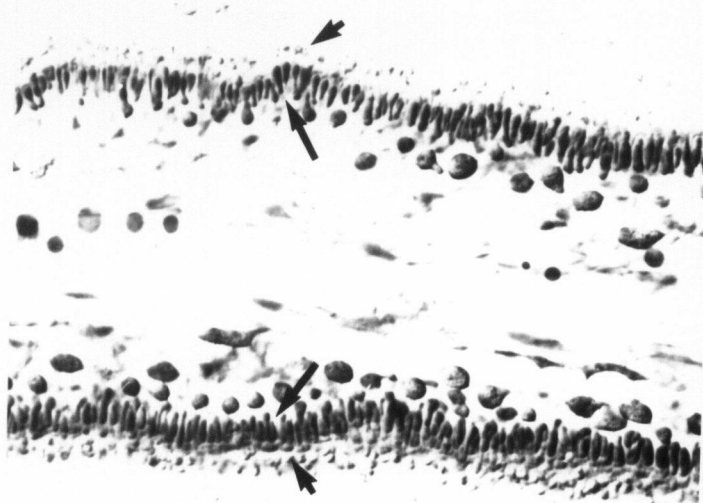
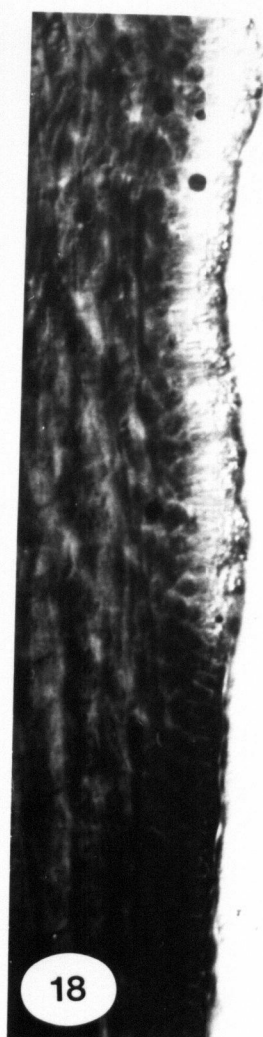
Figs. 6-11. *Gelidium coulteri* vegetative morphology. Fig. 6. habit of infertile plant, scale bar=5 mm; Fig. 7. Apex, apical cell domed and protruding, whole mount, scale bar=20 μ m; Fig. 8. Attachment pads from prostrate axis, whole mount, scale bar=50 μ m; Fig. 9. Vegetative upright axis showing cortex, medulla and rhizines (arrowhead), transverse section, scale bar=50 μ m; Fig. 10. Vegetative, upright axis, secondary pit-connections (arrows) between inner cortical and medullary cells, rhizines, longitudinal section, scale bar=20 μ m; Fig. 11. Outer cortical cells unoriented, isodiametric, whole mount, scale bar=20 μ m. Figs. 6, 11 Kirby Pt., Diana Is.; Fig. 7 east side Denman Is.; Figs. 8-10 Haines Is.



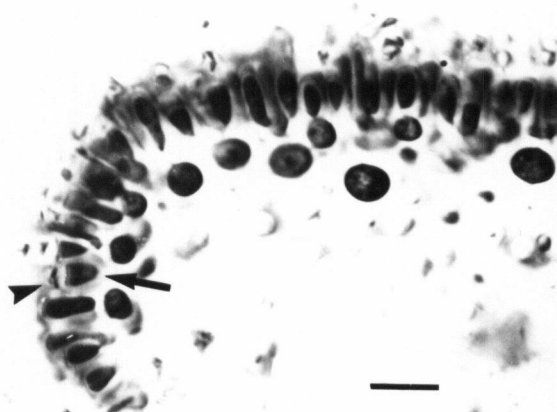
Figs. 12-17. *Gelidium coulteri* tetrasporophytes. Fig. 12. Habit with tetrasporangial stichidia (arrows), scale bar=5 mm; Fig. 13. Branched tetrasporangial stichidium, whole mount, scale bar=300 μm ; Fig. 14. Tetrasporangial stichidium with embedded tetrasporangia (arrows), whole mount, scale bar=50 μm ; Fig. 15. Undivided tetrasporangium with lateral pit connection (arrowhead), longitudinal section, scale bar=20 μm ; Fig. 16. Various ages of tetrasporangia, longitudinal section, scale bar=50 μm ; Fig. 17. Mature, cruciately divided tetrasporangium, transverse section, scale bar=20 μm ; Fig. 12 Kirby Pt., Diana Is.; Fig. 13 Meade Islets; Figs. 14-17 Galleon Pt., Hornby Is.



Figs. 18-20. *Gelidium coulteri* male gametophytes. Fig. 18. Spermatangial (arrow) and vegetative parts of branch, whole mount, scale bar=20 μm ; Fig. 19. Spermatangial mother cells (large arrows), spermatangia (small arrows) and spermatia (arrowheads), longitudinal section, scale bar=10 μm ; Fig. 20. Spermatangial mother cells (arrow) with periclinal wall cutting off spermatangium (arrowhead), transverse section, scale bar=10 μm . Figs. 18-20 Plants cultured from tetraspores, source: east side Denman Is.



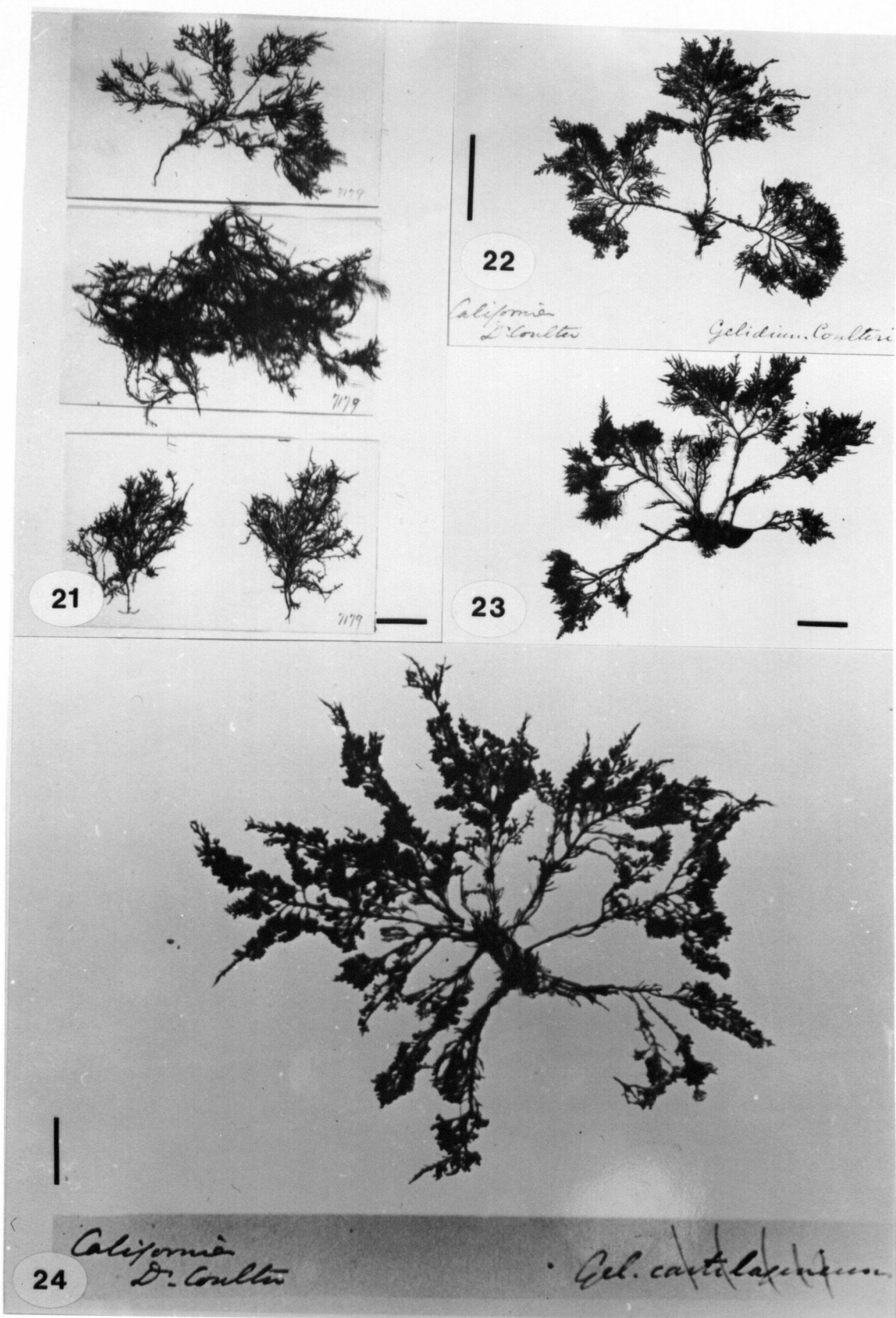
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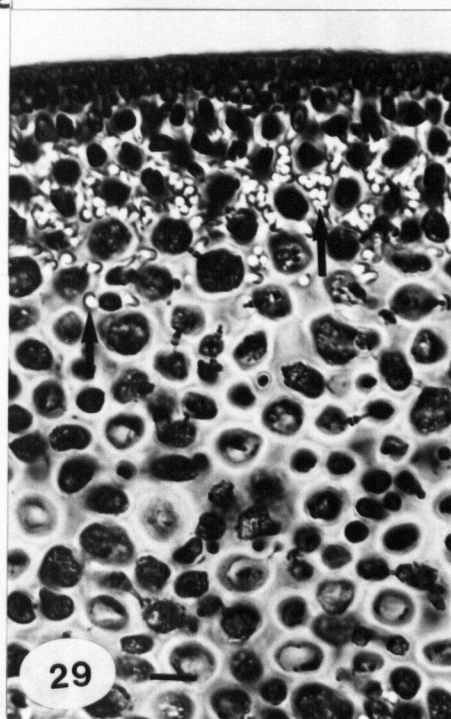
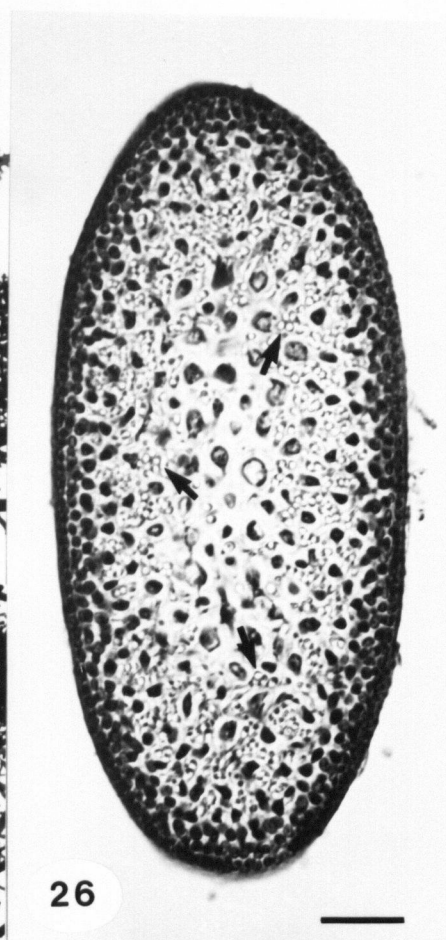
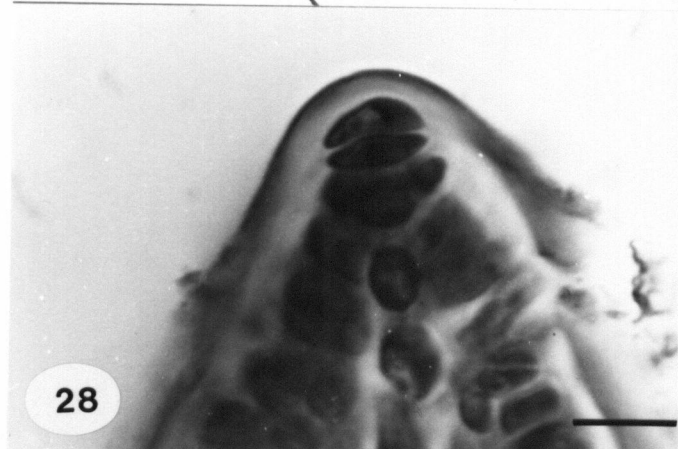
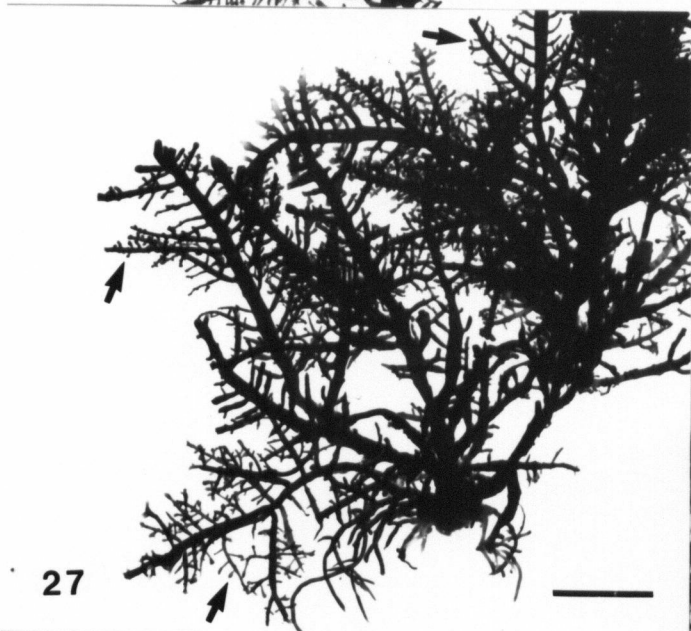
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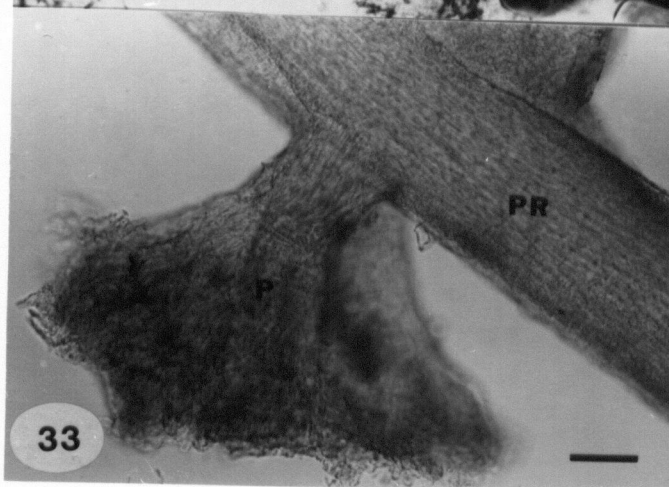
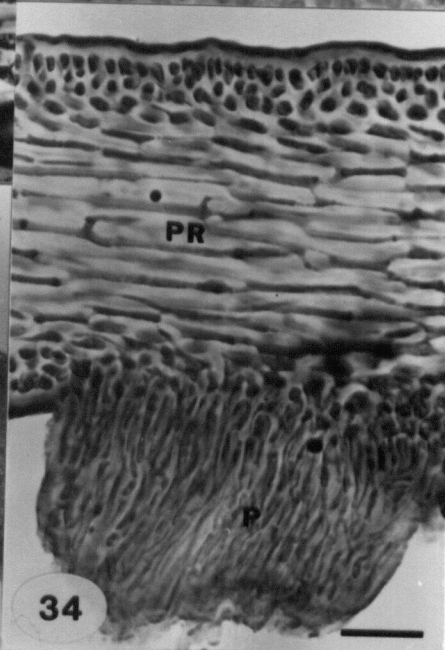
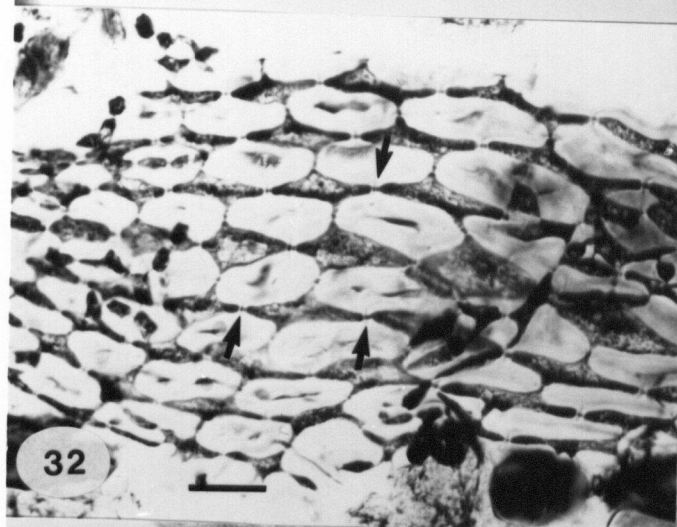
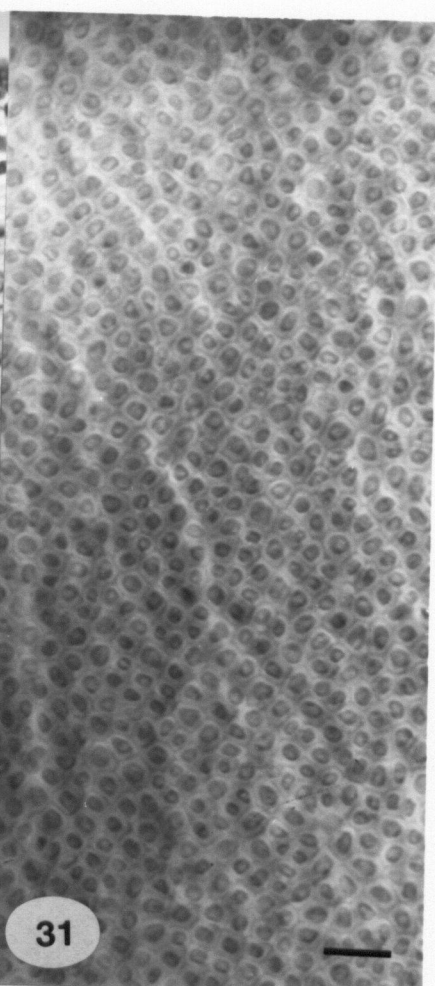
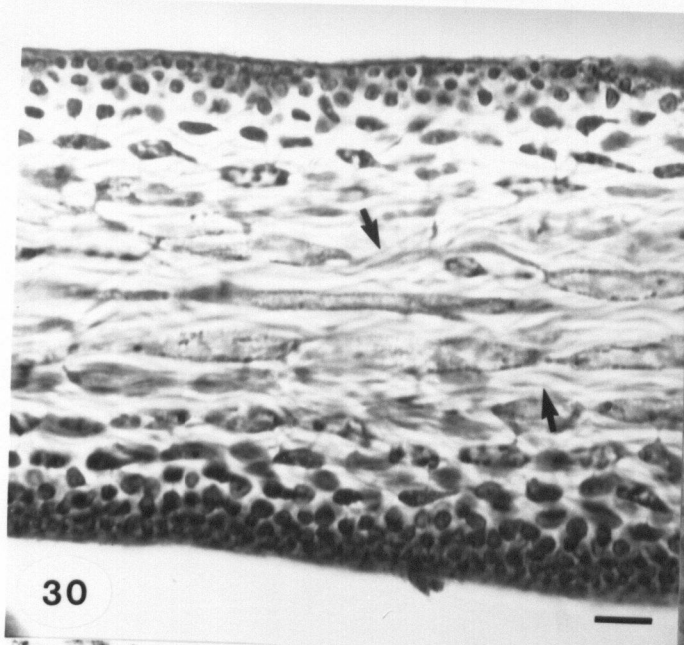
Figs. 21-24. *Gelidium coulteri* habit. Fig. 21. Herbarium specimens UC 494898, tetrasporophyte, Gardner's collection (#7179) from same tidepool as *G. sinicola* holotype, Point Cavalo, Marin Co., California, scale bar=20 μ m; Fig. 22. Isotype in TCD, tetrasporophyte, scale bar=20 mm; Fig. 23. Isotype, AHFH 53929, scale bar=10 mm; Fig. 24. Reproduction of photograph of lectotype in TCD, scale bar=10 mm.



Figs. 25-29. *Gelidium purpurascens* habit and vegetative morphology. Fig. 25. Habit of part of tetrasporophyte, scale bar=10 mm; Fig. 26. Vegetative branch showing cortex, medulla and rhizines (arrows), transverse section, scale bar=50 μ m; Fig. 27. Habit of basal part of tetrasporophyte showing prostrate axes and tetrasporangial stichidia (arrows), scale bar=10 mm; Fig. 28. Apex of female gametophyte branch, apical cell domed, longitudinal section, scale bar=10 μ m; Fig. 29. Vegetative branch, rhizines (arrows) in inner cortex/outer medulla transverse section, scale bar=20 μ m. Figs. 25, 27, 29 Meade Islets, Fig. 26 Orlebar Pt., Gabriola Is., Fig. 28 Kirby Pt., Diana Is.

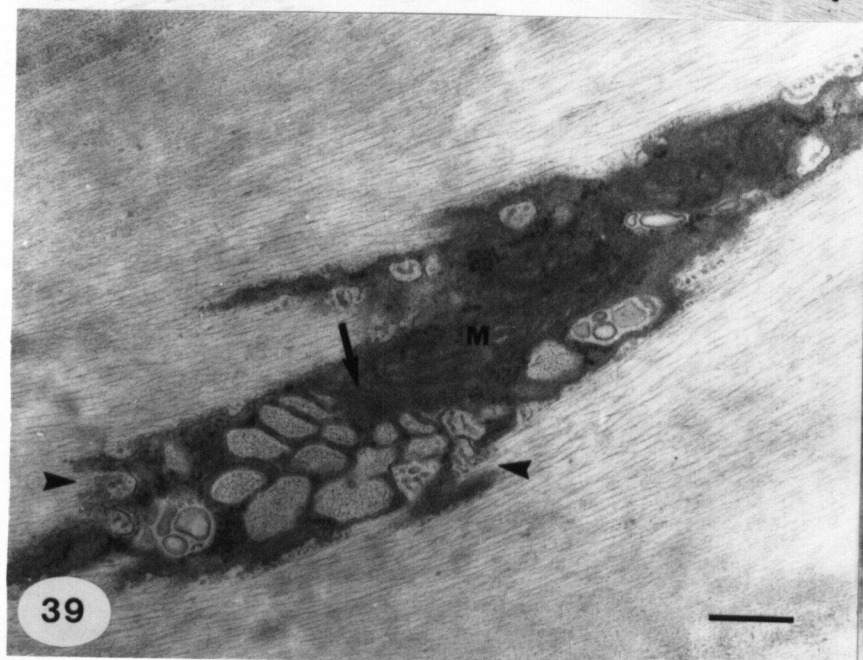
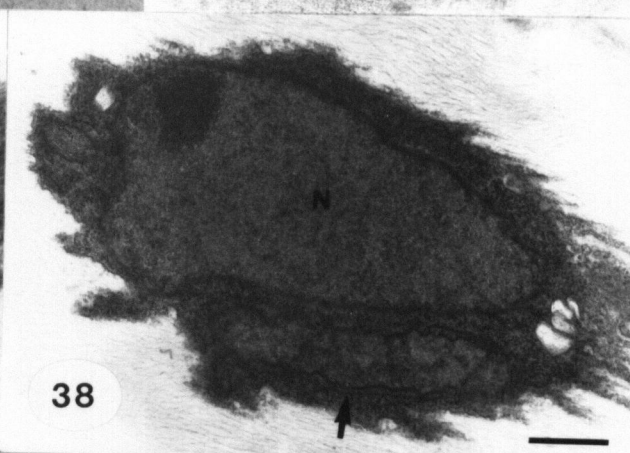
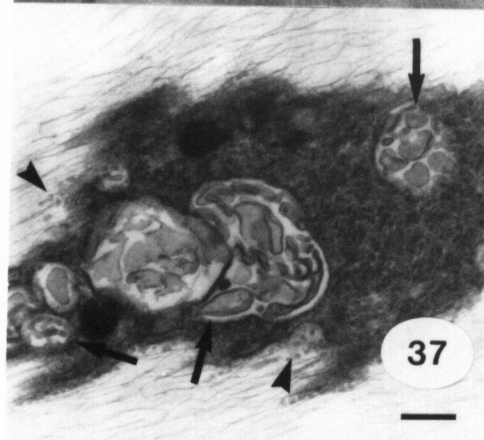
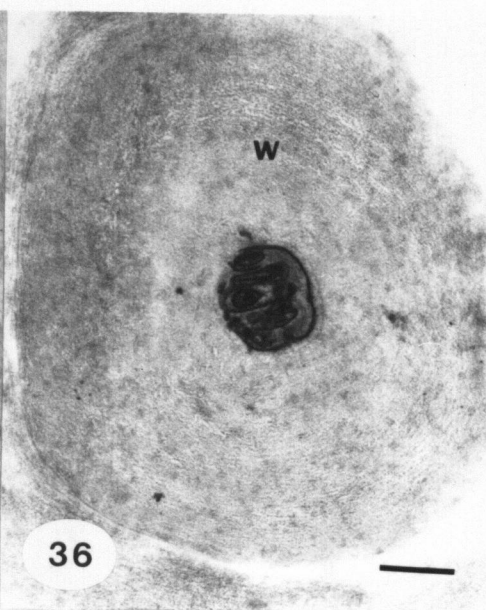
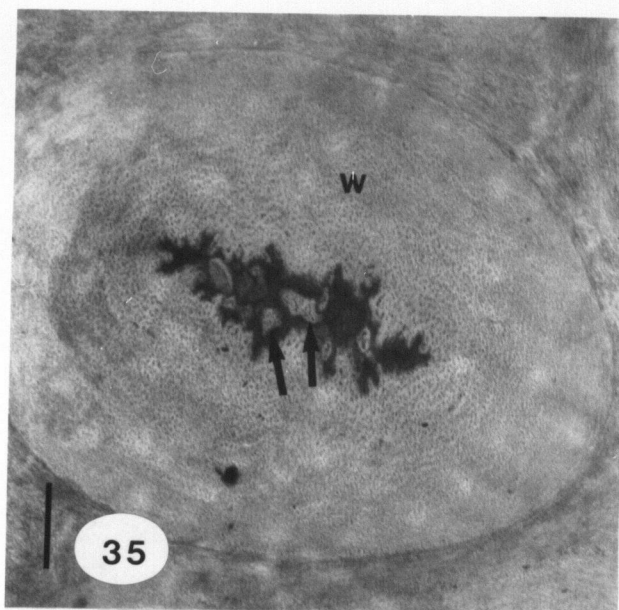


Figs. 30-34. *Gelidium purpurascens* vegetative morphology. Fig. 30. Vegetative branch with cortex, medulla and rhizines (arrows), longitudinal section, scale bar=20 μm ; Fig. 31. Surface cortical cells isodiametric, unoriented, equidistant, whole mount, scale bar=20 μm ; Fig. 32. Medullary cells of second order plate interconnected by secondary pit connections (arrows), squashed longitudinal section, scale bar=30 μm ; Fig. 33. Attachment pad on prostrate axis, whole mount, scale bar=100 μm ; Fig. 34. Attachment pad formed from elongate cortical cells, longitudinal section of prostrate axis, scale bar=50 μm . Fig. 30 Orlebar Pt., Gabriola Is., Figs. 31, 32 east side Denman Is., Figs. 33, 34 Whalebone Bay, Gabriola Is.

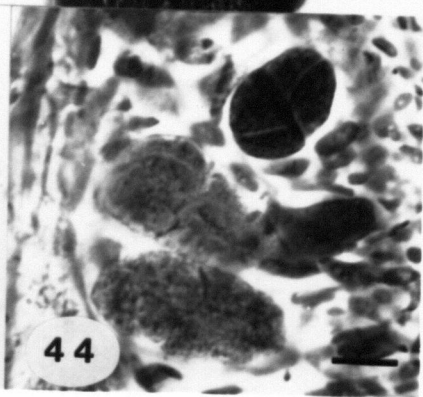
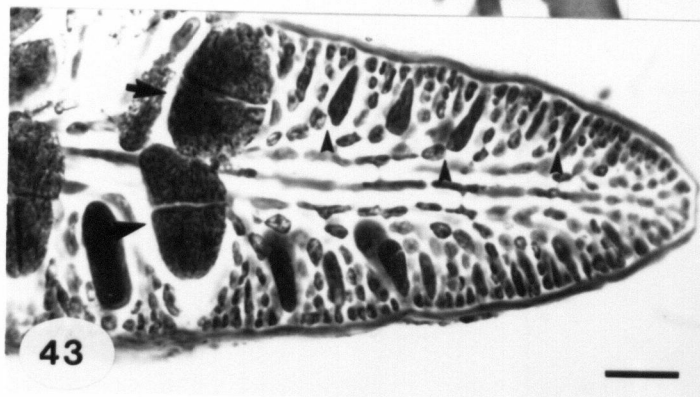
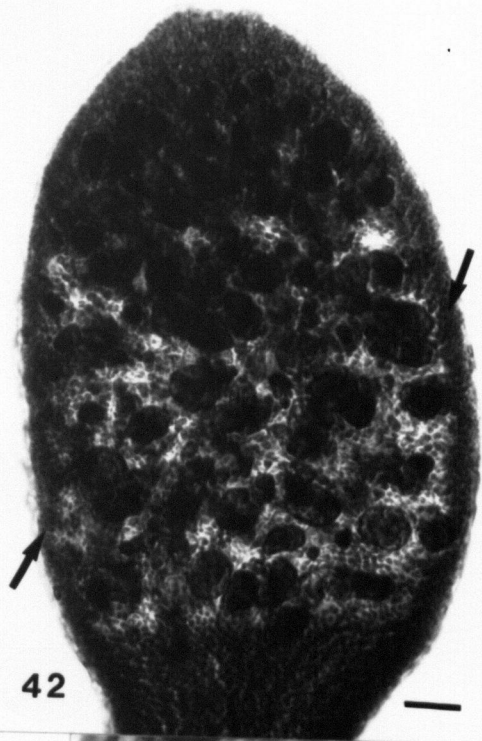
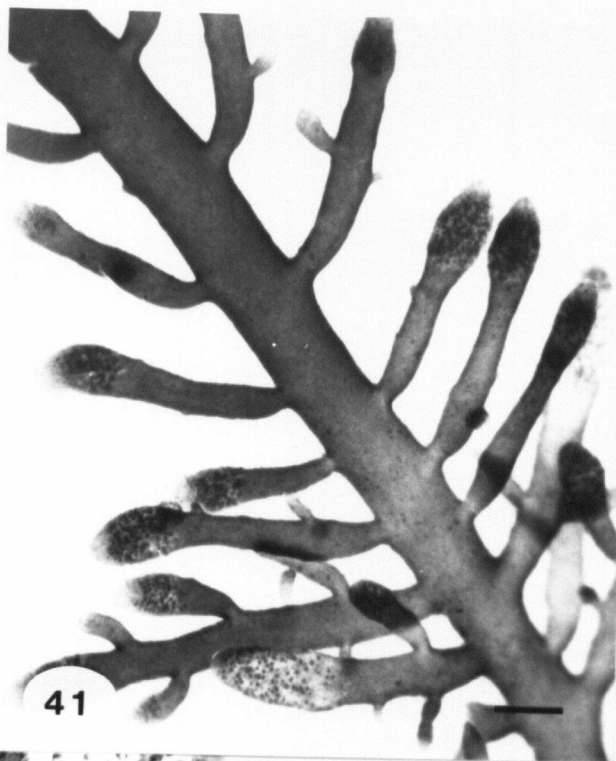


Figs. 35-40. Ultrastructure of rhizines in *Gelidium purpurascens* and *G. vagum*.

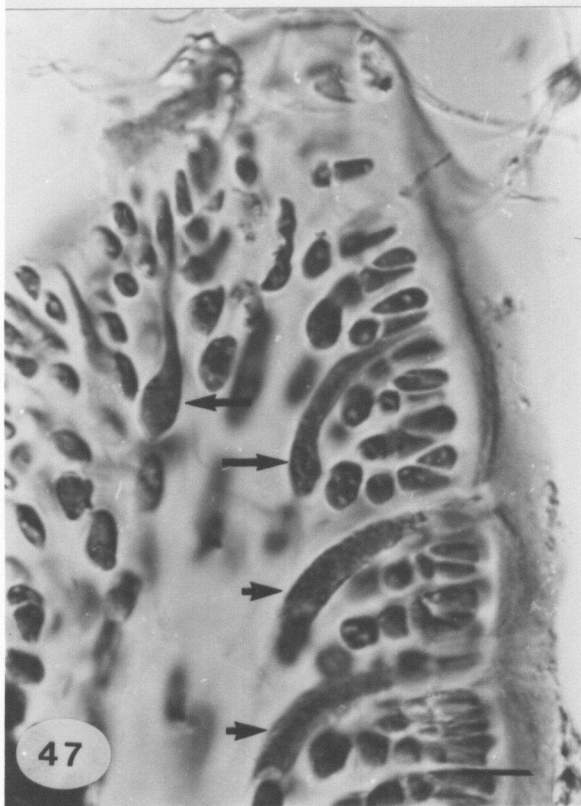
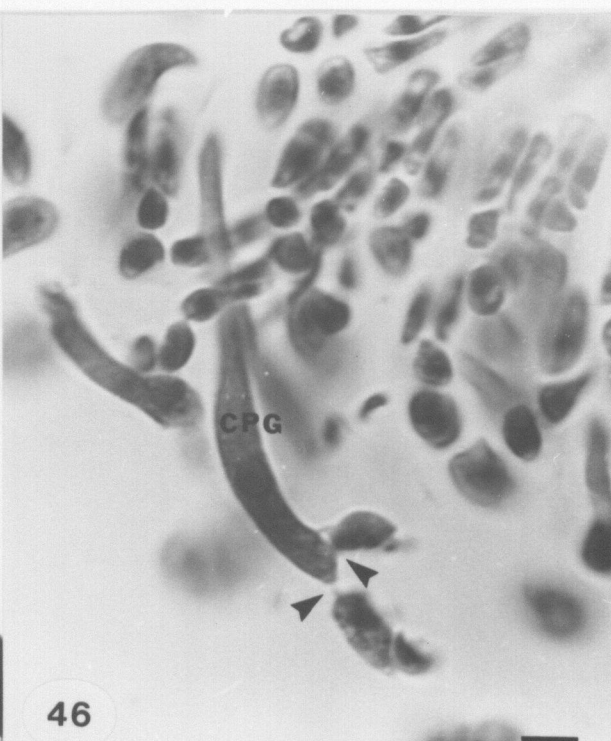
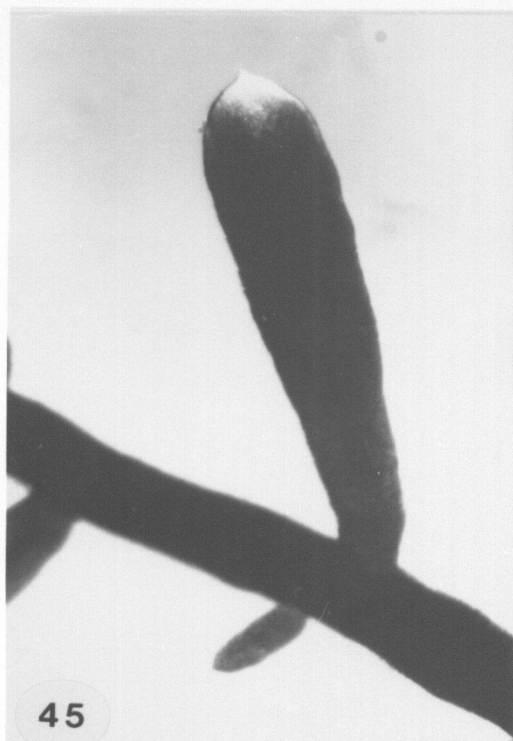
Figs. 35, 36, 39, 40. *Gelidium purpurascens*. Fig. 35. Young rhizines with convoluted plasma membrane, large vesicles with fibrillar contents (arrows), thick wall, transverse section, scale bar=500 nm; Fig. 36. Old rhizine with smooth plasma membrane profile, cytoplasm vesiculate, membranes thickened, very thick wall, transverse section, scale bar=500 nm; Figs. 37, 38. *Gelidium vagum*. Fig. 37. Young rhizine with vesicles containing vesicular and tubular contents (arrows), some tubular bodies in wall outside indented plasma membrane (arrowheads), longitudinal section, scale bar=200 nm; Fig. 38. Part of young rhizine with nucleus and chloroplast (arrow) with inner encircling thylakoid but lacking inner parallel thylakoids, longitudinal section, scale bar=500 nm; Fig. 39. Young rhizine with dictyosome (arrow) producing vesicles with fibrillar contents and releasing contents to wall (at arrowheads), elongate mitochondrion (M), longitudinal section, scale bar=400 nm; Fig. 40. Old rhizine, cytoplasm vesiculate, membranes thickened, plasma membrane profile smooth, longitudinal section, scale bar=400 nm; Figs. 35, 39 Orlebar Pt., Gabriola Is., Figs. 36, 40 culture source: Whalebone Bay, Gabriola Is., Figs. 37, 38 culture source: Tribune Bay, Hornby Is.



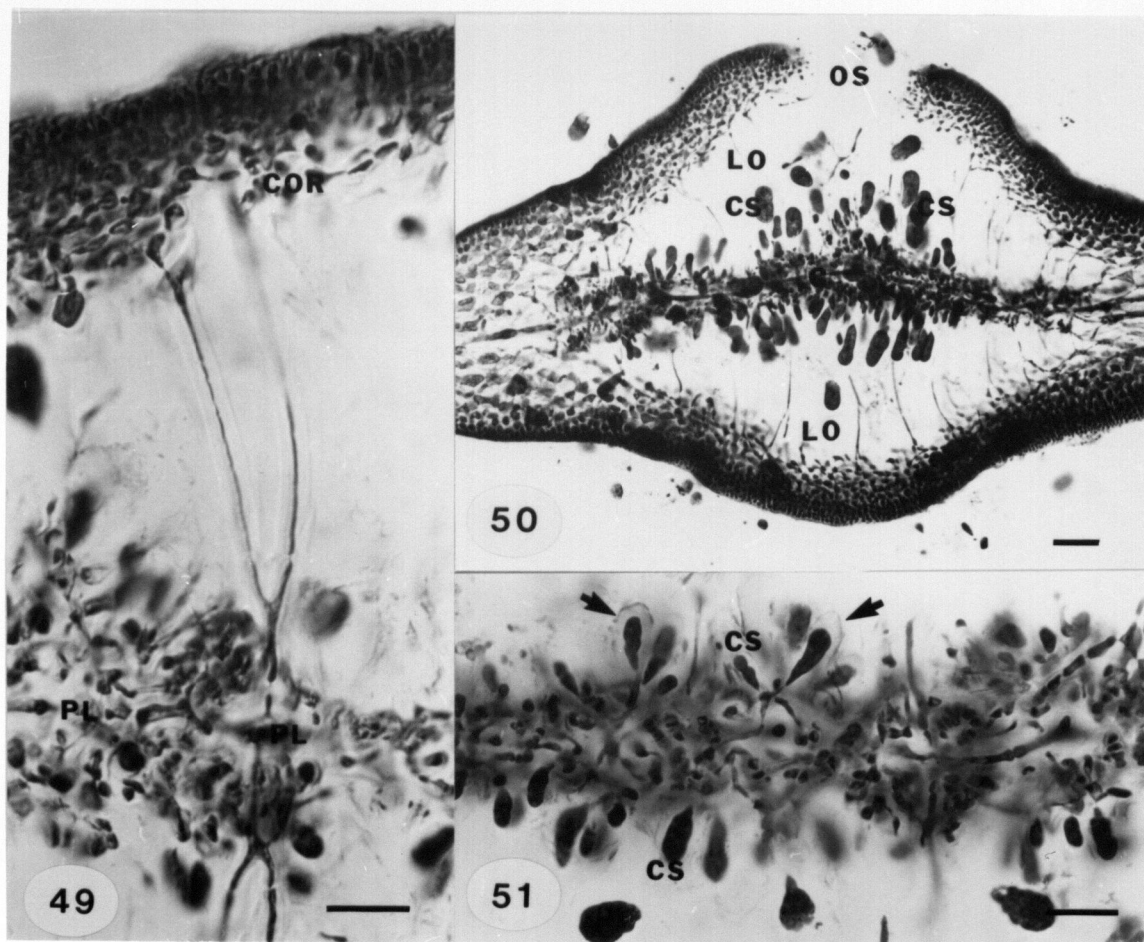
Figs. 41-44. *Gelidium purpurascens* tetrasporophytes. Fig. 41. Tetrasporangial stichidia, habit, scale bar=500 μm ; Fig. 42. Tetrasporangial stichidium with narrow sterile margin (arrow), whole mount, scale bar=50 μm ; Fig. 43. Stichidial branch apex, young tetrasporangia with basal pit connections (arrowheads) and maturing tetrasporangia with one periclinal division (arrows), longitudinal section, scale bar=50 μm ; Fig. 44. Cruciately divided tetrasporangium, transverse section, scale bar=20 μm . Figs. 41, 44 Orlebar Pt., Gabriola Is., Fig. 42 Meade Islets, Fig. 43 Cape Suspiro, Alaska.



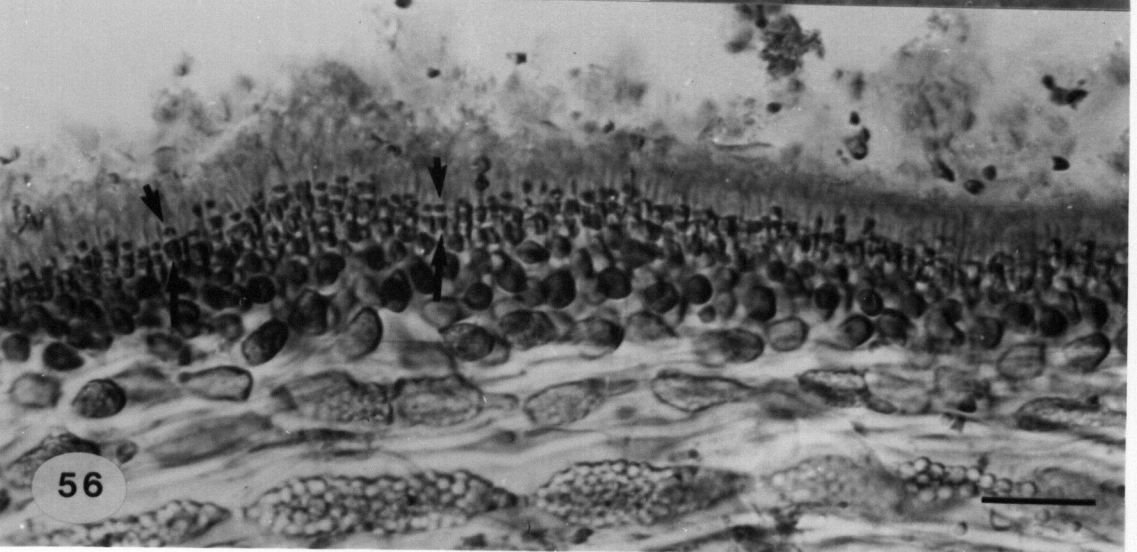
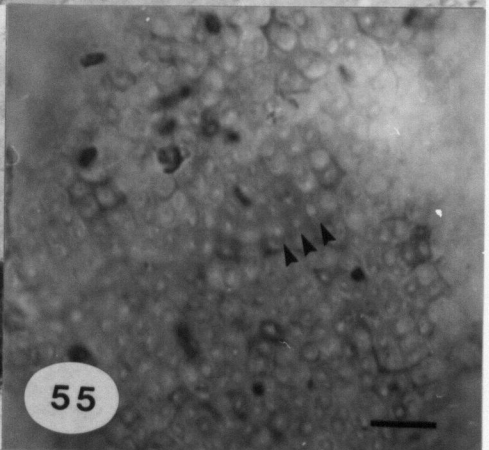
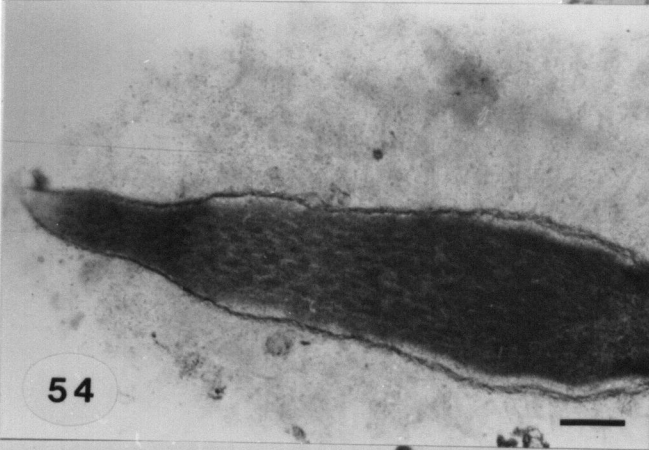
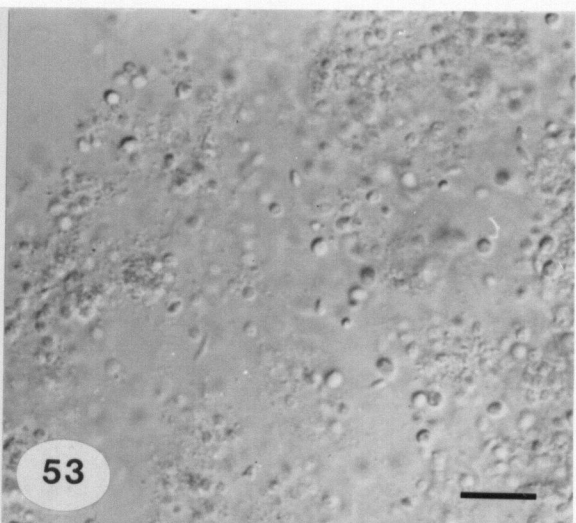
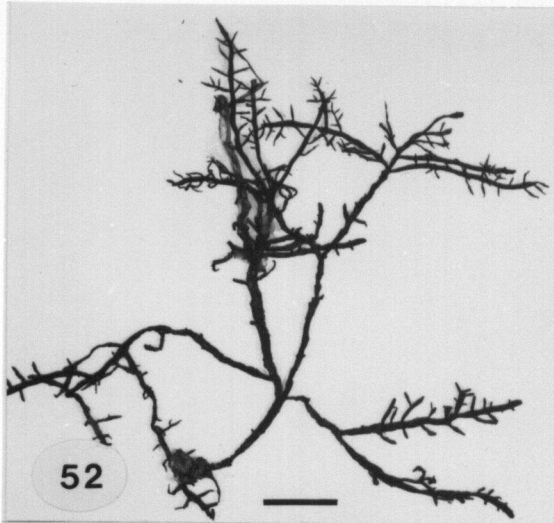
Figs. 45-48. *Gelidium purpurascens* female gametophytes. Fig. 45. Carpogonial stichidium, habit, scale bar=100 μm ; Fig. 46. Intercalary carpogonium, with two pit connections (arrowheads), squashed longitudinal section, scale bar=5 μm ; Fig. 47. Apex with intercalary carpogonia (all arrows), most proximal carpogonia sessile (large arrows), longitudinal section, scale bar=400 μm ; Fig. 48. Cystocarps single and in series on branch, habit, scale bar=2 mm. Fig. 45 Whalebone Bay, Gabriola Is., Fig. 46 Geer Islets, Figs. 47, 48 Kirby Pt., Diana Is.



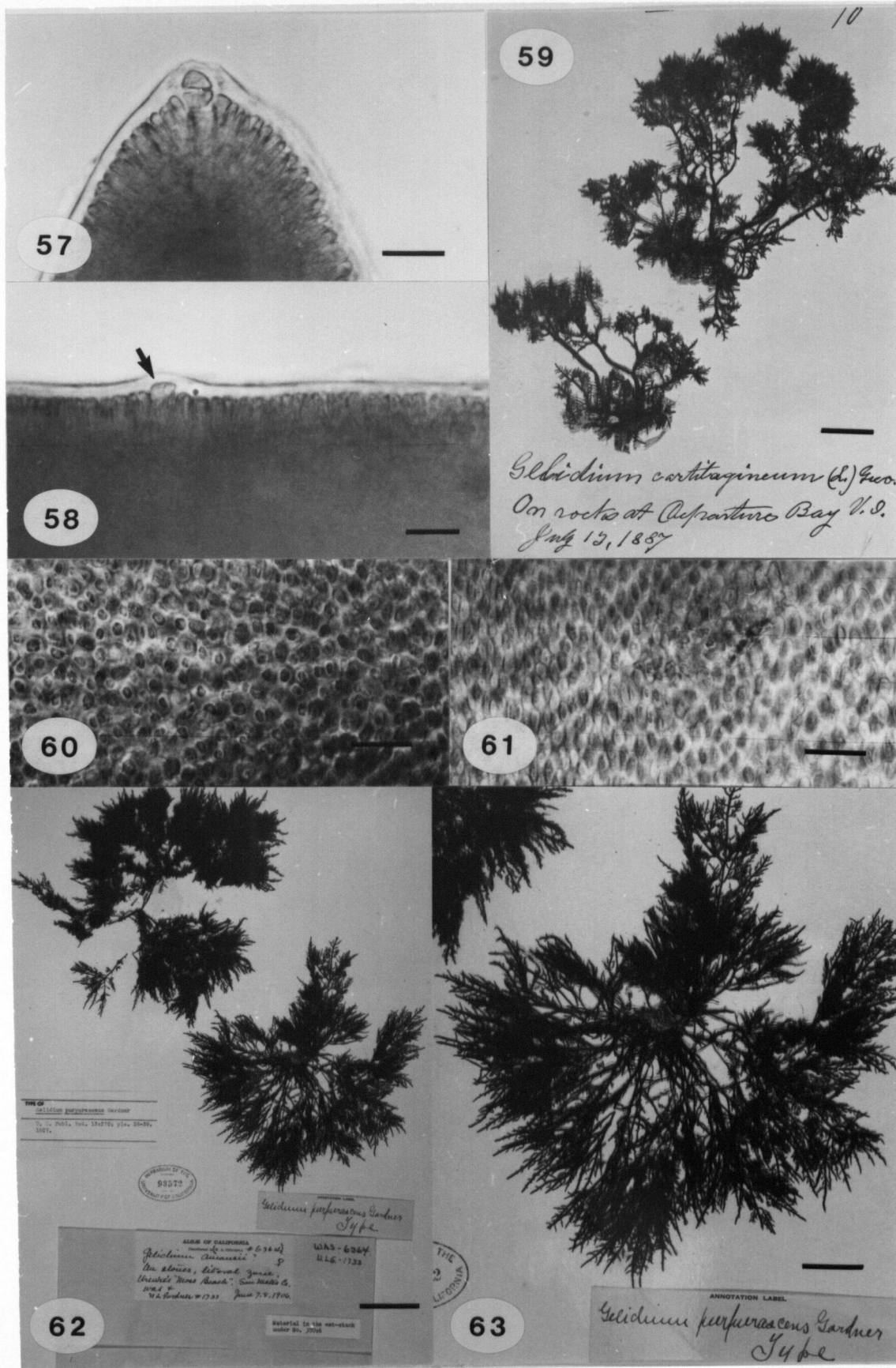
Figs. 49-51. *Gelidium purpurascens* carposporophytes. Fig. 49. Stretched third order filament between second order plate and cystocarp cortex, longitudinal section, scale bar=10 μm ; Fig. 50. Mature cystocarp with two locules, carposporophyte concentrated around second order plate and producing carposporangia, longitudinal section through ostiole of upper locule, scale bar=50 μm ; Fig. 51. Detail of carposporophyte, young carposporangia with mucilage coating (arrows), longitudinal section, scale bar=25 μm . Figs. 49, 51 Geer Islets, Fig. 50 Kirby Pt., Diana Is.



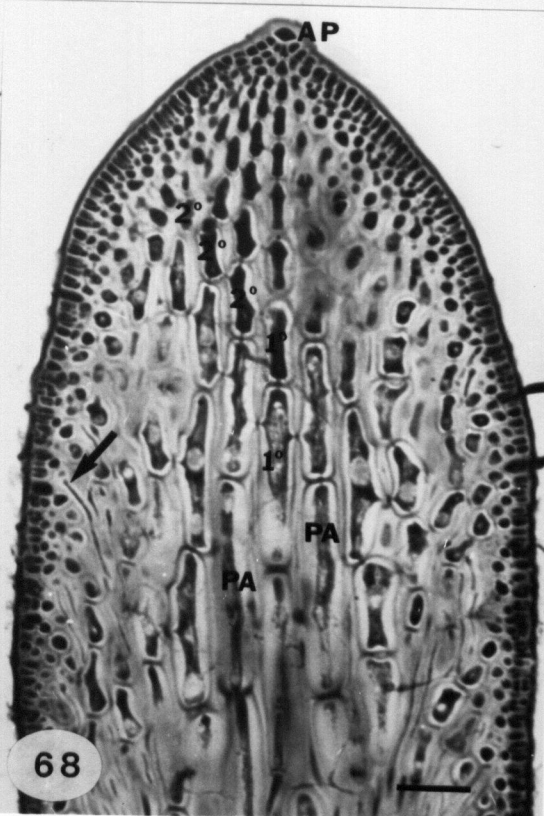
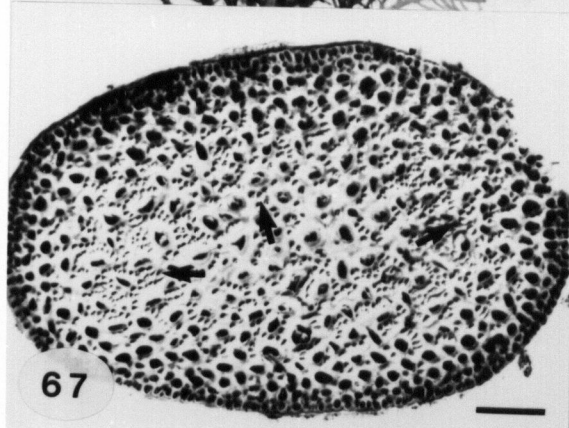
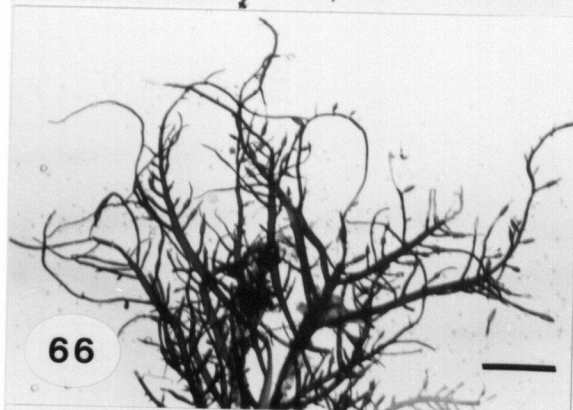
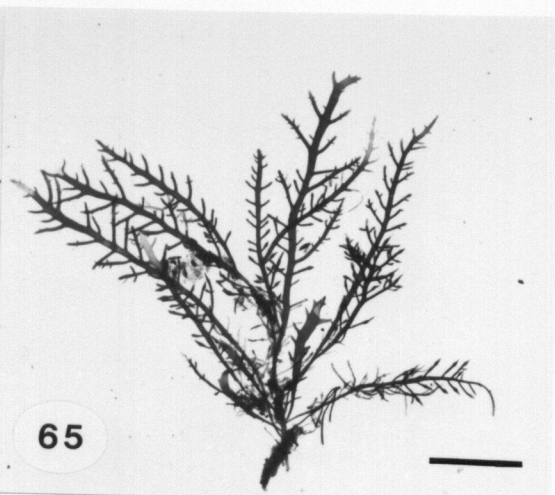
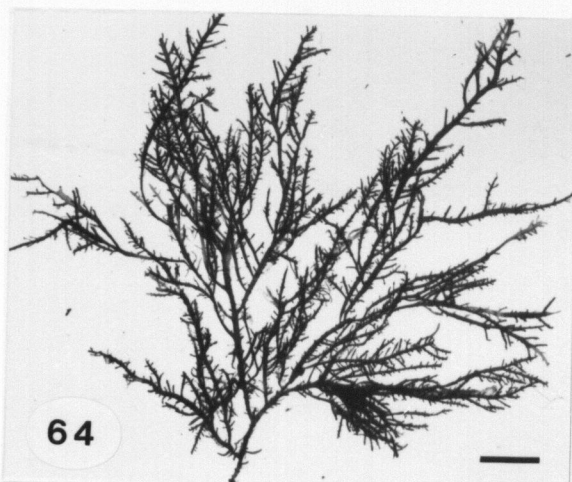
Figs. 52-56. *Gelidium purpurascens* male gametophytes. Fig. 52. Habit, whole mount, scale bar=5 mm; Fig. 53. Spermatia, whole mount, scale bar=20 μm ; Fig. 54. Spermatangial stichidium with thickened and ruptured thallus wall over spermatangia, whole mount, scale bar=100 μm ; Fig. 55. Holes in wall (arrowheads) through which spermatia have been released, whole mount, scale bar=10 μm ; Fig. 56. Spermatangial mother cells (large arrows) and spermatangia (small arrows) cut off by periclinal wall, longitudinal section, scale bar=10 μm . Fig. 52 Nootka Sound UBC A53657, Figs. 53, 54, 56 cultured from tetraspores, source: Whalebone Bay, Gabriola Is., Fig. 55 Esteban Pt., Vancouver Is. UBC A10813.



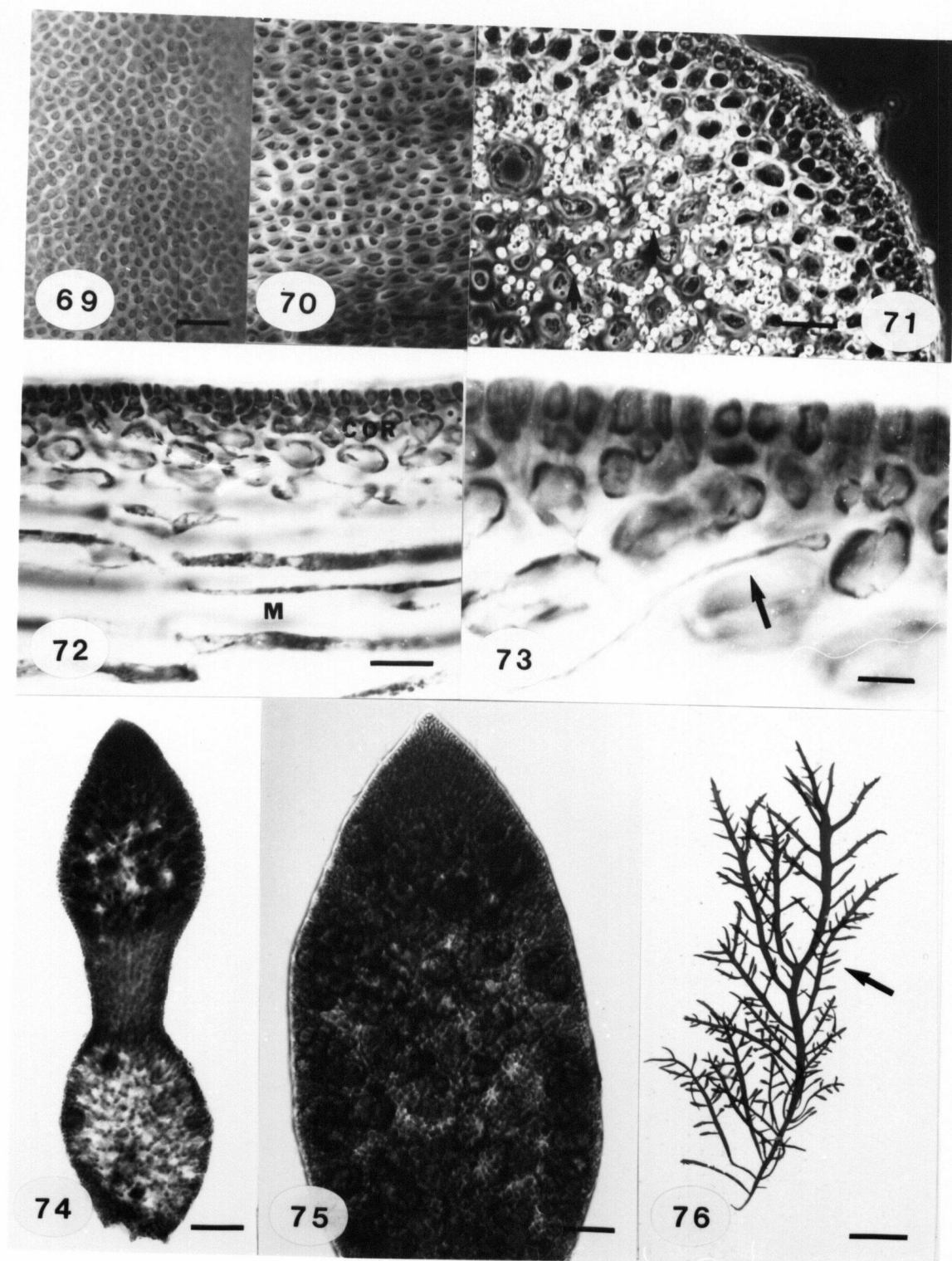
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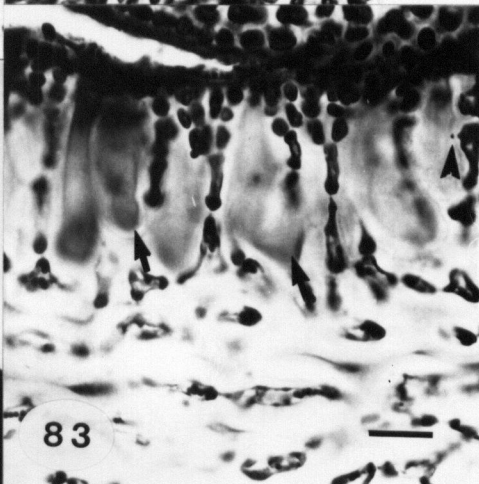
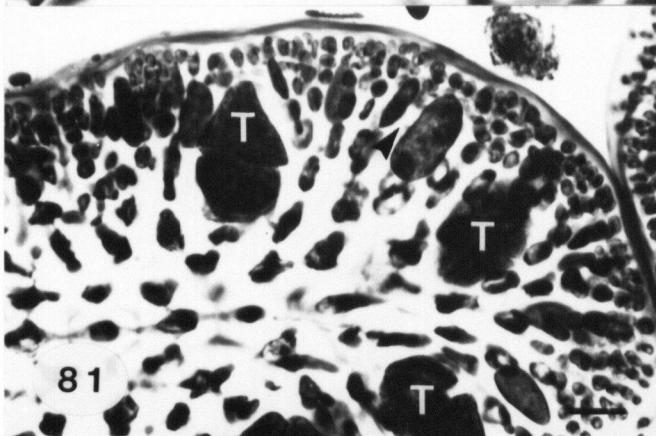
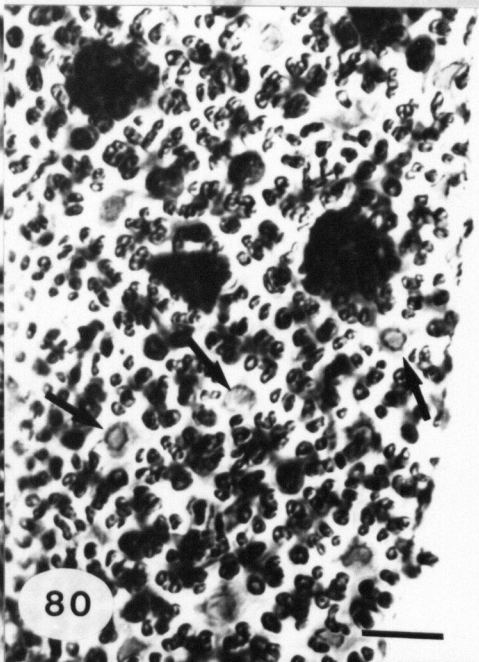
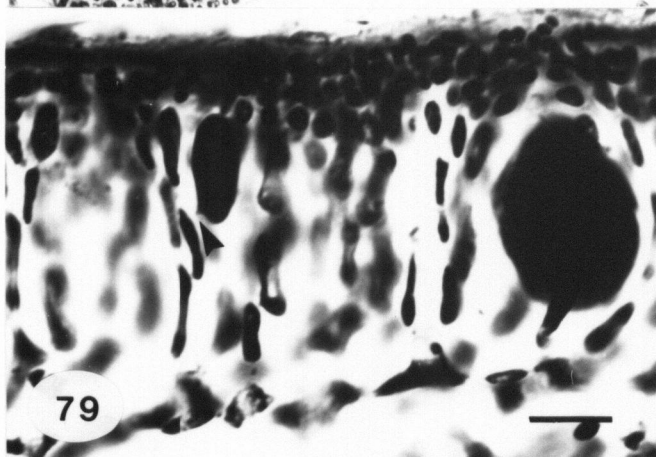
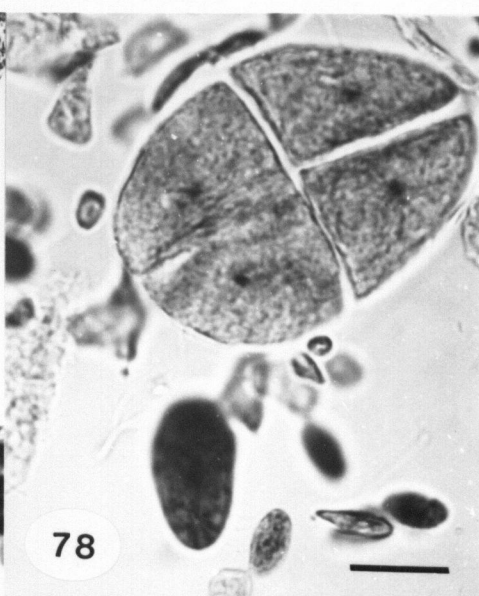
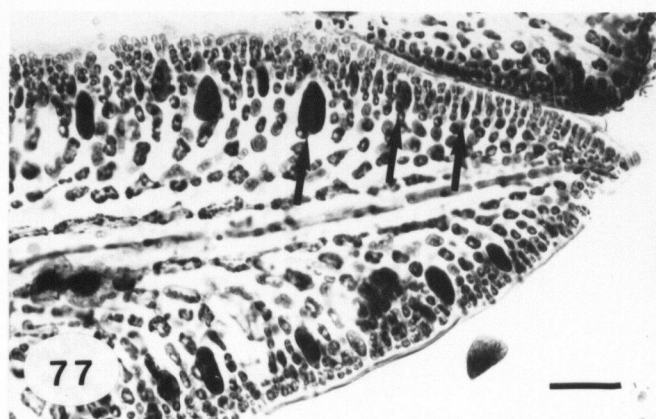
Figs. 64-68. *Gelidium vagum* habit and vegetative morphology. Fig. 64. Habit of gametophyte, scale bar=10 mm; Fig. 65. Habit of tetrasporophyte, scale bar=10 mm; Fig. 66. Habit of gametophyte with whip-like ultimate branches, scale bar=10 mm; Fig. 67. Vegetative branch with cortex, medulla and rhizines (arrows), transverse section, scale bar=25 μm ; Fig. 68. Apical organization in vegetative branch, apical cell cuts off cells of axial file/first order filament, periaxial cells cut off second order filament, rhizines (arrow) cut off inner cortical cells, longitudinal section in plane of second order plate, scale bar=50 μm . Figs. 64-68 Tribune Bay, Hornby Is.



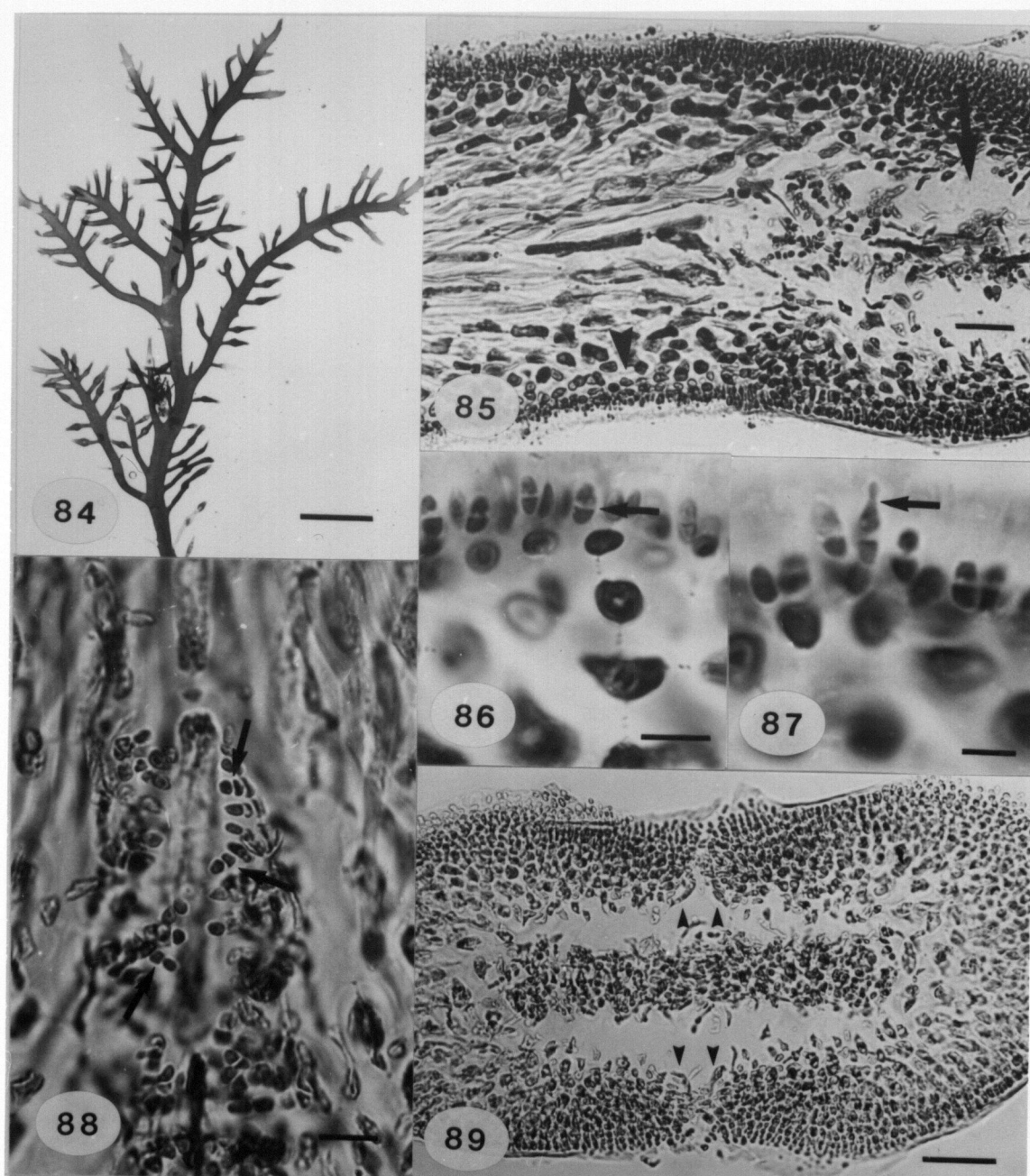
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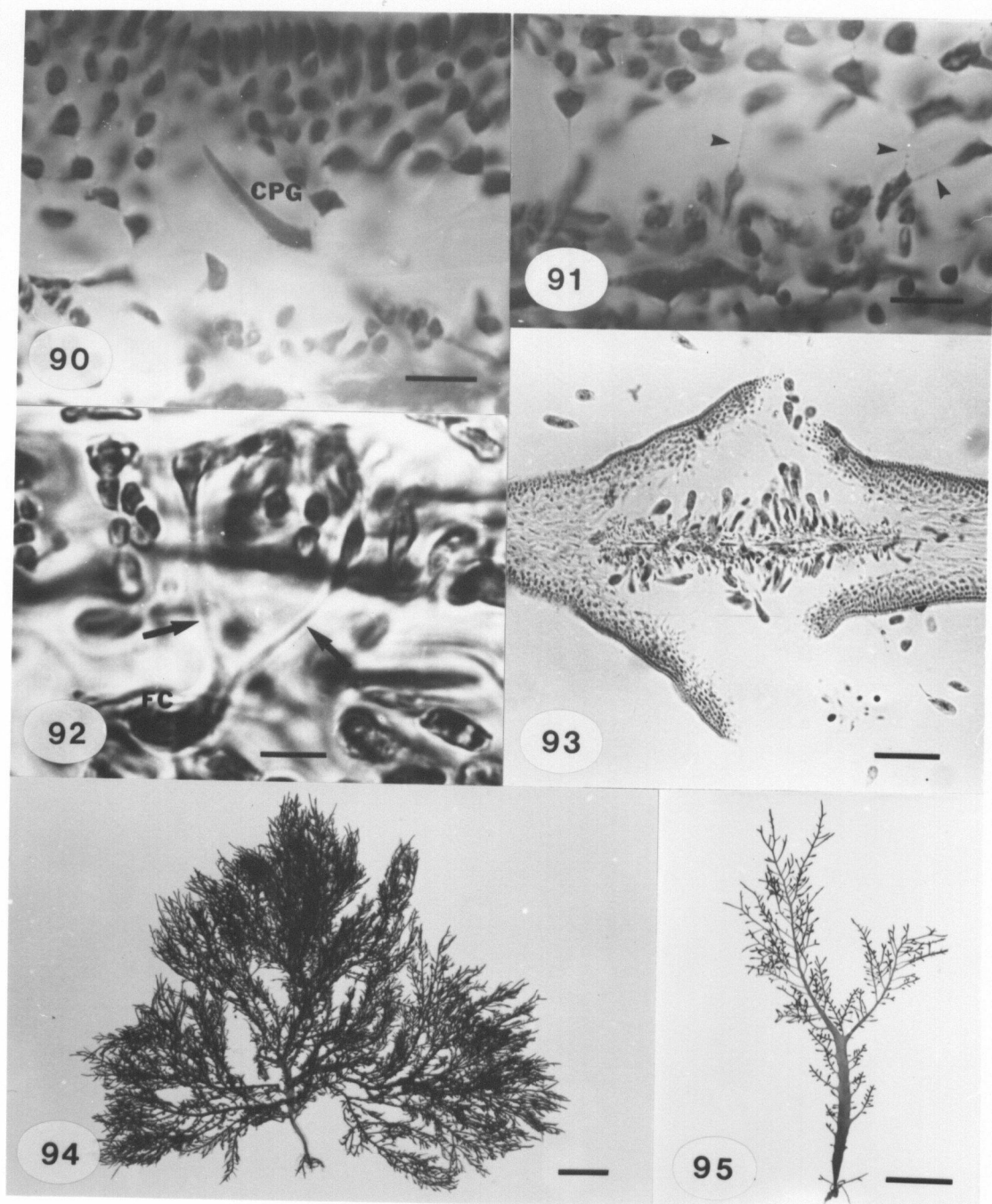
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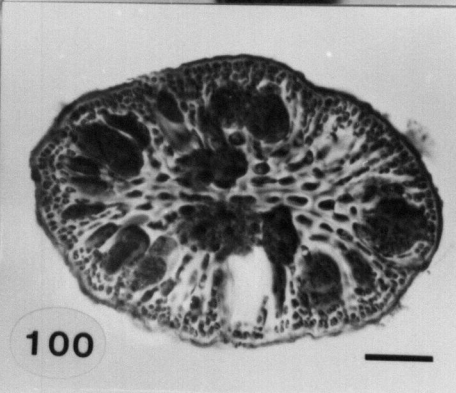
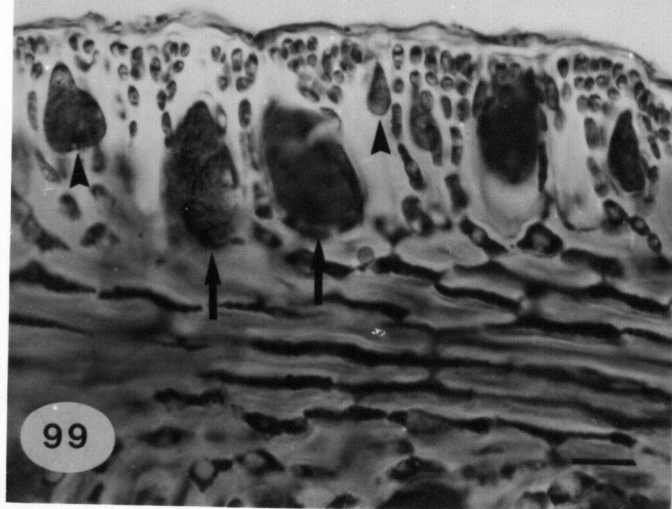
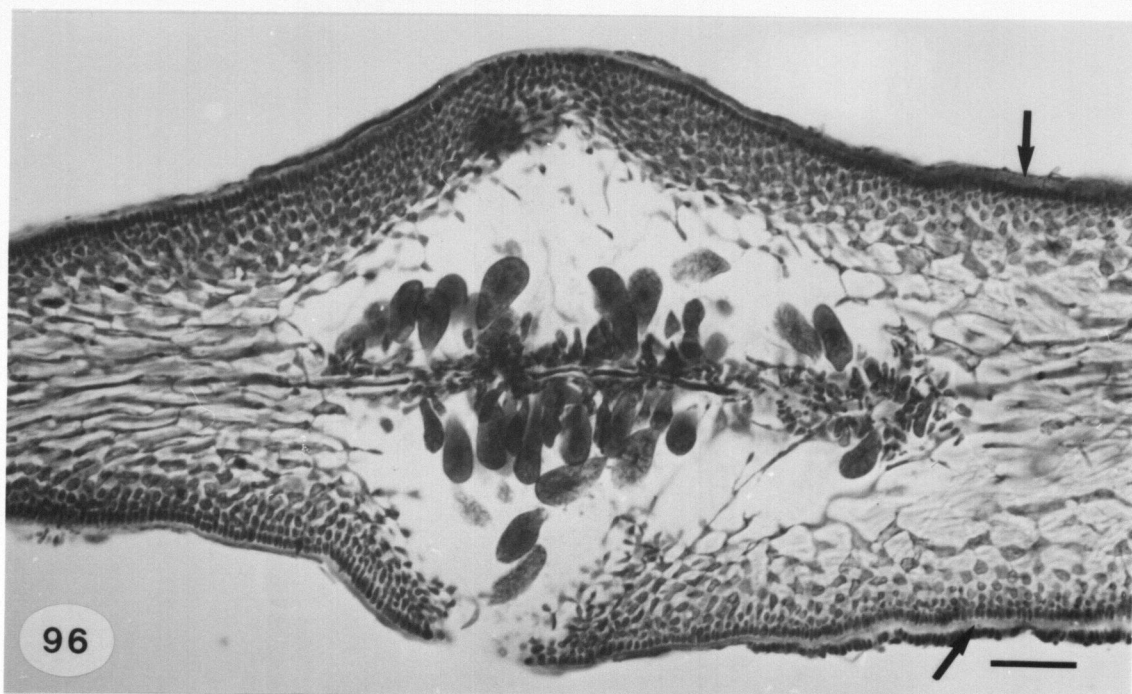
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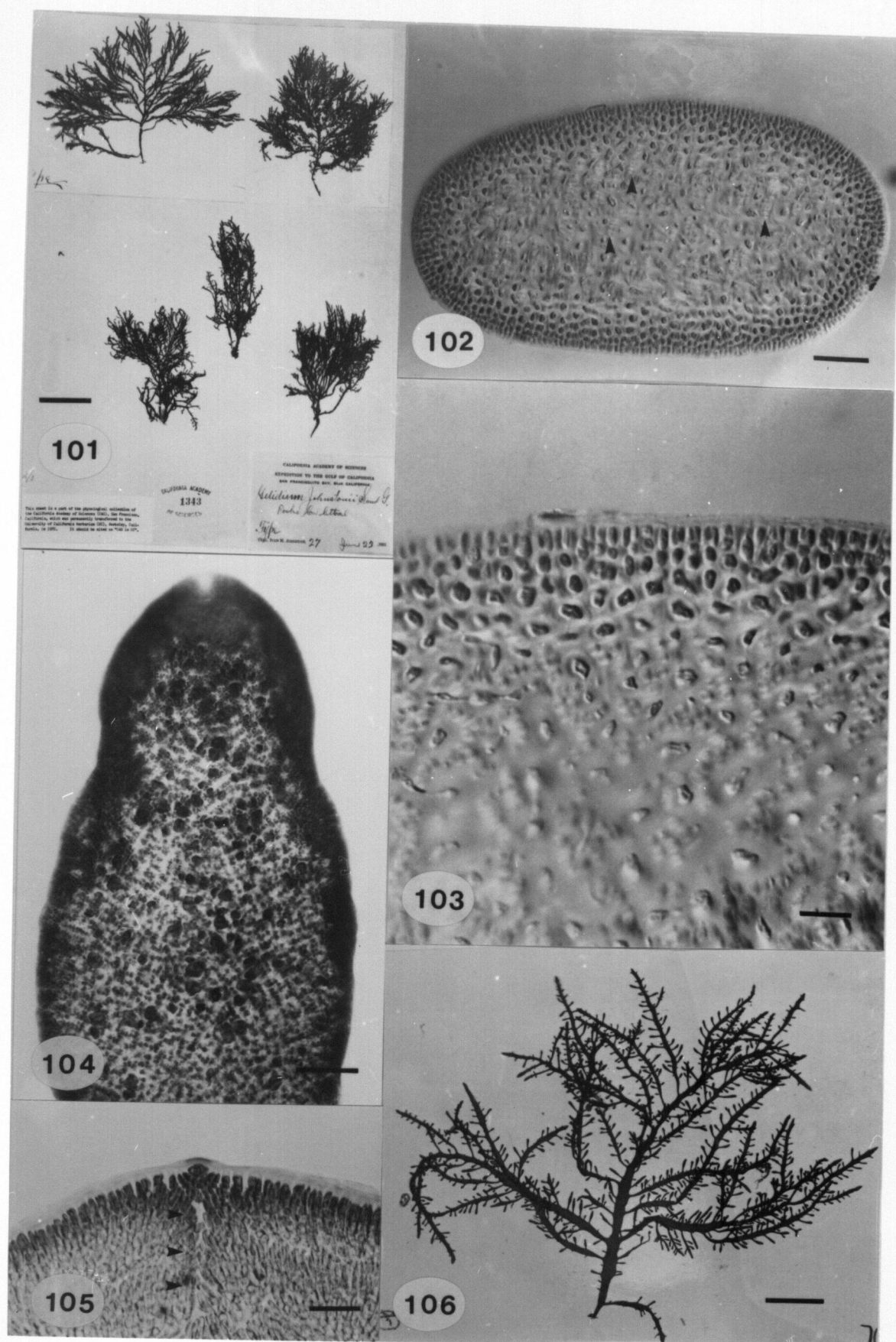


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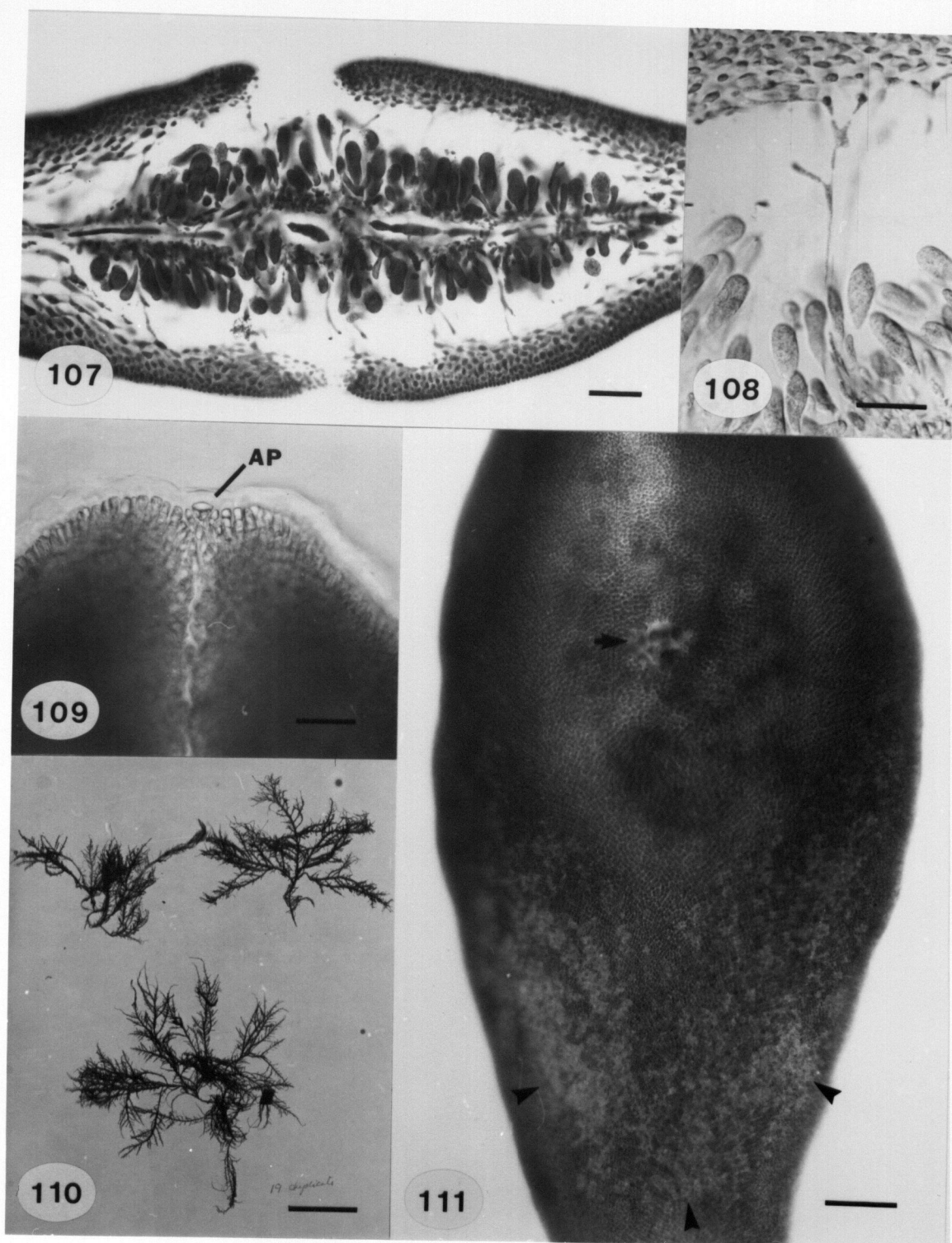


Figs. 101-106. *Gelidium johnstonii* habit, vegetative and reproductive morphology.

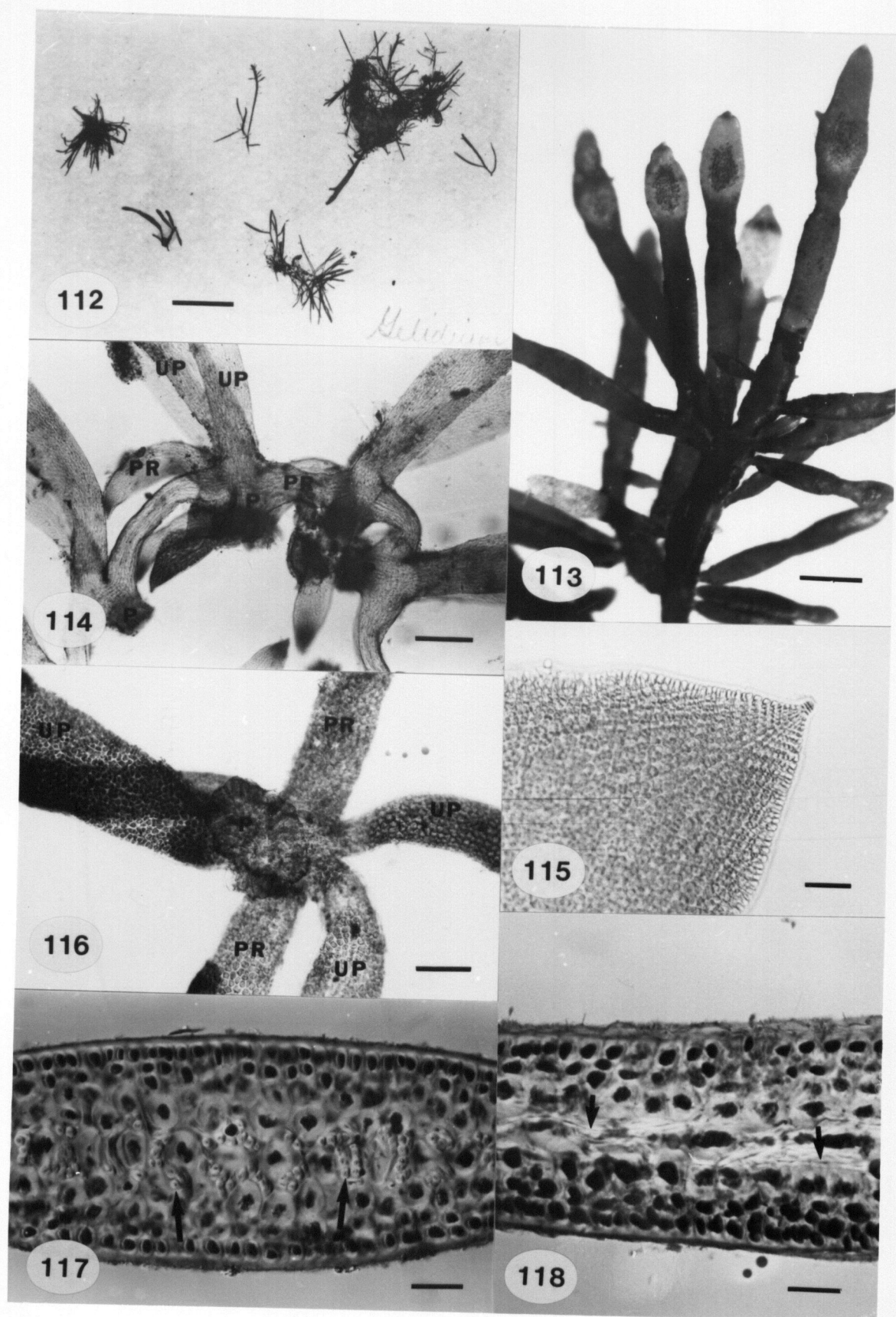
Fig. 101. Habit of holotype, CAS 1343 in UC, tetrasporophytes, Bahia San Francisquito, Gulf of California, Baja California del Norte, Mexico, scale bar=40 mm; Fig. 102. Vegetative branch with cortical cells in anticlinal rows, medulla and rhizines (arrowheads), transverse section, scale bar=50 μ m; Fig. 103. Cortical cells in anticlinal rows, rhizines between inner cortical and medullary cells, transverse section, scale bar=100 μ m; Fig. 104. Tetrasporangial stichidium with sterile margin, whole mount, scale bar=20 μ m; Fig. 105. Apical cell and furrow (arrowheads) of tetrasporophyte branch, whole mount, scale bar=30 μ m; Fig. 106. Habit of tetrasporophyte with stichidia, scale bar=10 mm. Figs. 104, 105 Puerto Escondido, Gulf of California, Baja California del Sur, Mexico, Fig. 106 Punta Perico, Gulf of California, Mexico.



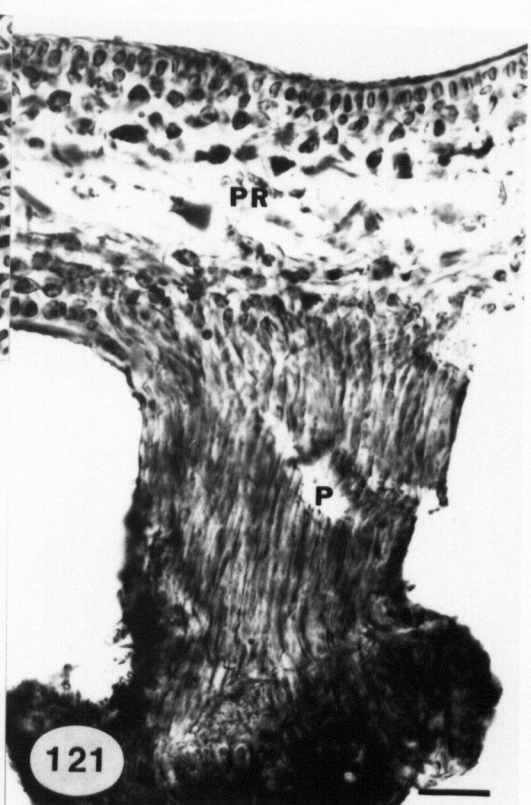
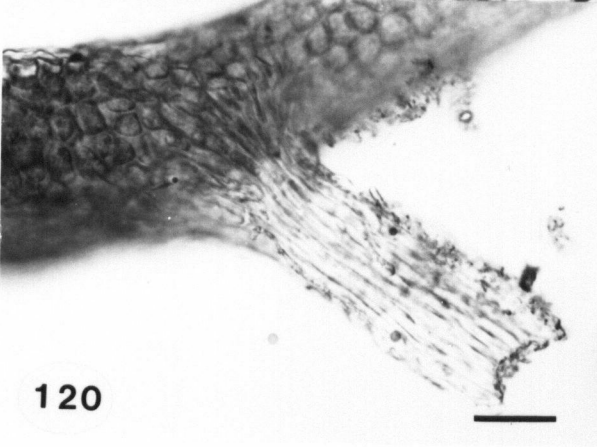
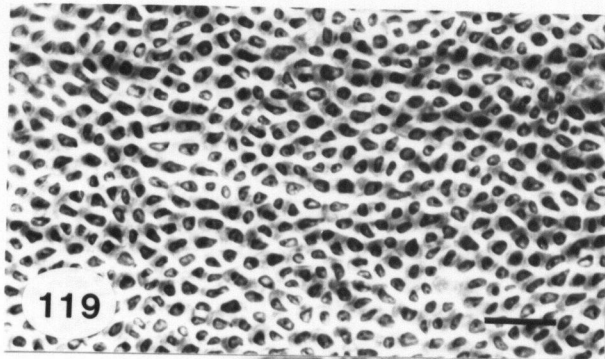
Figs. 107-109, 111. *Gelidium johnstonii* reproductive morphology. Fig. 110. *G. vagum* habit. Fig. 107. Mature cystocarp, longitudinal section, scale bar=50 μm ; Fig. 108. Stretched third order filament in cystocarp between second order plate and pericarp, longitudinal section, scale bar=40 μm ; Fig. 109. Apex of gametangial stichidium, note apical furrow and apical cell slightly recessed between cortical lobes, whole mount, scale bar=50 μm ; Fig. 110. *Gelidium vagum* habit of drift plant from Ladysmith, B.C., UBC A64965, scale bar=40 μm ; Fig. 111. Mature cystocarp (top) with ostiole (arrow) and adjacent proximal (bottom) spermatangial area (arrowheads), whole mount, scale bar=100 μm . Fig. 107 Puerto Escondido, Gulf of California Baja California del Sur, Mexico, AHFH 50299, Fig. 108 Punta Escondido, Gulf of California, Baja California del Sur, Mexico, Dawson #7170, Fig. 109 Ensenada Bocochibampo, Sinaloa, Mexico, AHFH 4179, Fig. 111 Bahia Aqua Verde, Gulf of California, Baja California del Sur, Mexico, AHFH 502677.



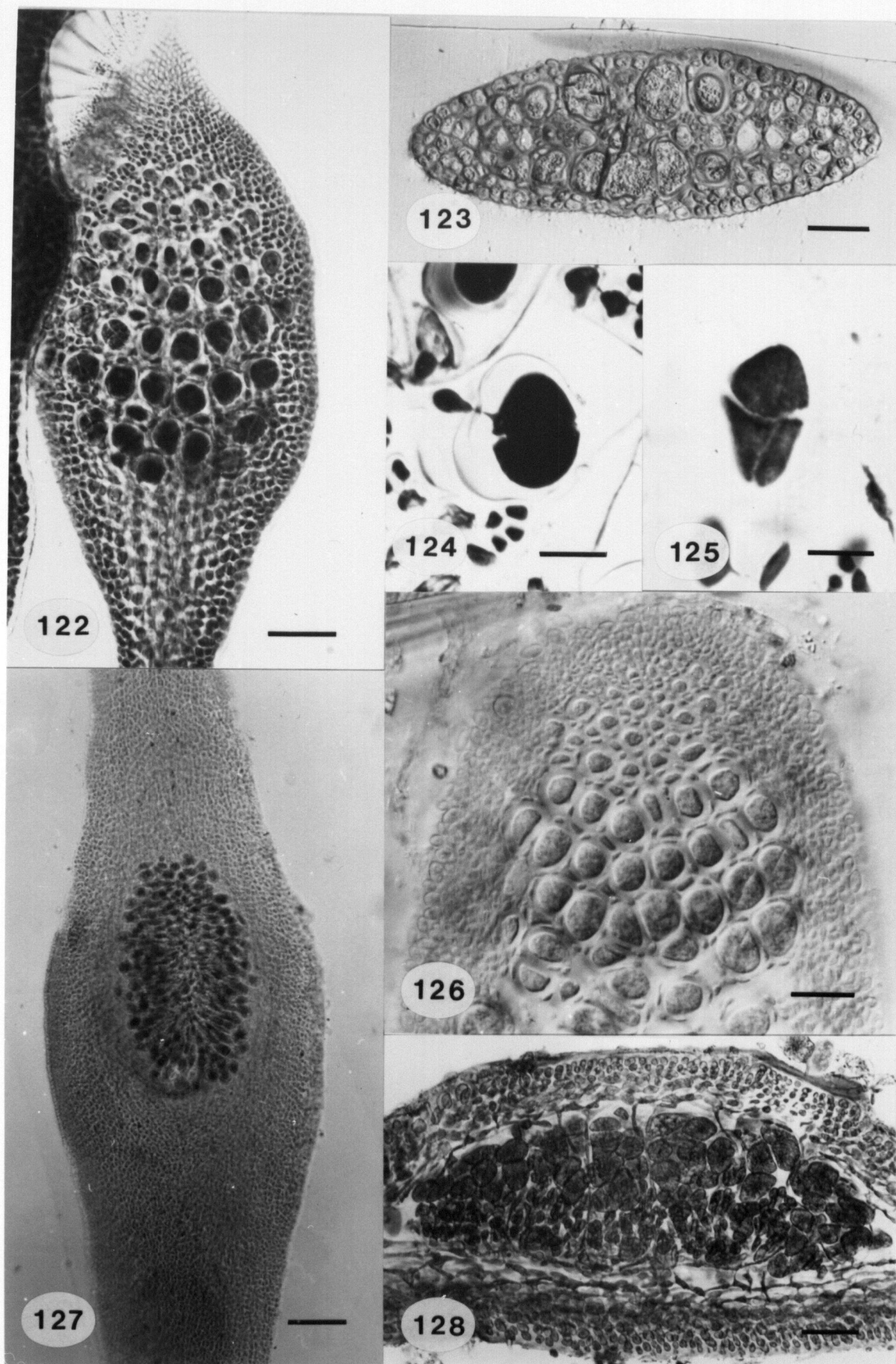
Figs. 112-118. *Pterocladia caloglossoides* habit and vegetative morphology. Fig. 112. Habit, scale bar=10 mm; Fig. 113. Habit of upright axes, branches perpendicular to main axis, tetrasporangial, scale bar=150 μm ; Fig. 114. Habit of prostrate axes, several upright branches and attachment pad at a node, scale bar=700 μm ; Fig. 115. Apex of vegetative branch, apical initial and lateral initial (upper left corner) domed and protruding, files of cells in V's, whole mount, scale bar=100 μm ; Fig. 116. Bottom view of flared attachment pad on prostrate axis and four radiating upright branches, whole mount, scale bar=100 μm ; Fig. 117. Upright axis with cortex, medulla and rhizines (arrows), transverse section, scale bar=30 μm ; Fig. 118. Vegetative axis with narrow cortex, medulla and rhizines (arrows), transverse section, scale bar=30 μm . Fig. 112 Sea Otter Sound, Alaska, Fig. 113 Orlebar Pt., Gabriola Is., Fig. 114 Barra de Navidad, Jalisco, Mexico, Dawson #3733 slide #1327 (in AHFH) Figs. 115-118 Georgina Pt., Mayne Is.



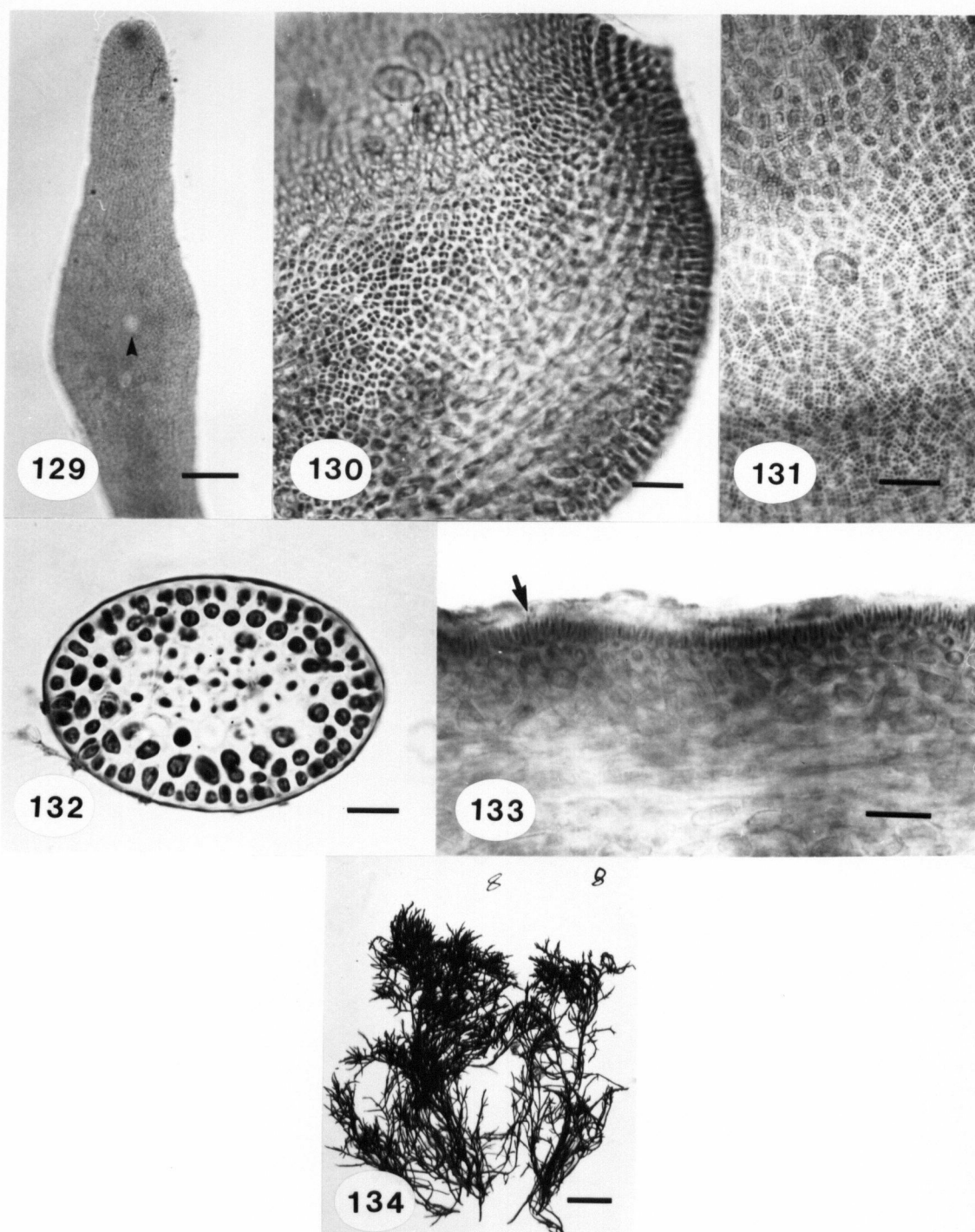
Figs. 119-121. *Pterocladia caloglossoides* vegetative morphology. Fig. 119. Surface cortical cells oval, equidistant, unoriented, whole mount, scale bar=50 μm ; Fig. 120. Attachment pad on prostrate axis, composed of elongated cortical cells, whole mount, scale bar=30 μm ; Fig. 121. Attachment pad on prostrate axis composed of elongated cortical cells, longitudinal section, scale bar=40 μm . Figs. 119-121 Georgina Pt., Mayne Is.



Figs. 122-128. *Pterocladia caloglossoides* reproductive morphology. Fig. 122. Tetrasporangial stichidium, tetrasporangia in V's, whole mount, scale bar=100 μm ; Fig. 123. Tetrasporangial stichidium, transverse section, scale bar=40 μm ; Fig. 124. Tetrasporangium with lateral pit connection, squash preparation, scale bar=20 μm ; Fig. 125. Mature, cruciately divided tetrasporangium, squash preparation, scale bar=20 μm ; Fig. 126. Tetrasporangia in V's in Dawson's material (Dawson #8593 slide 1177) from Guadalupe Is., Baja California del Norte, Mexico, whole mount, scale bar=100 μm ; Fig. 127. Cystocarp in Dawson's material (Dawson #8593 slide 1177) from Guadalupe Is., Baja California del Norte, Mexico, whole mount, scale bar=40 μm ; Fig. 128. Cystocarp with single locule, Dawson's material from Barra de Navidad, Jalisco, Mexico, Dawson #3733 (wet preserved in AHFH), longitudinal section, scale bar=30 μm . Figs. 122-125 Georgina Pt., Mayne Is.



Figs. 129-133. *Pterocladia caloglossoides* reproductive morphology. Fig. 134. *Gelidium coulteri*. Fig. 129. Cystocarp with single ostiole (arrowhead), Dawson #8593 slide 1177, whole mount, scale bar=100 μm ; Fig. 130. Spermatangial area, Dawson #3733 slide 1325, whole mount, scale bar=20 μm ; Fig. 131. Spermatangia, Dawson #3733 slide 1325, whole mount, scale bar=40 μm ; Fig. 132. Vegetative branch with narrow medulla, Dawson #3733 slide 1326, transverse section, scale bar=20 μm ; Fig. 133. Spermatangia (arrow), Dawson #3733 slide 1325, optical section of whole mount, scale bar=30 μm ; Fig. 134. Habit of Macoun's collection of "*Gelidium crinale*" from Beacon Hill, Vicotria CANA 3474 (was CAN 207), scale bar=10 mm. Fig. 129 Guadalupe Is., Baja California del Norte, Figs. 130-133 Barra de Navidad, Jalisco, Mexico.



Figs. 135-140. Historical collections and type specimens. Fig. 135. *Gelidium purpurascens*, Macoun's collection as "*G. amansii*" from Departure Bay, CANA 4349 (was CAN 310), scale bar=20 mm; Fig. 136. *G. robustum*, collection from "Shoal B. Victoria", UBC A7861, scale bar=50 mm; Fig. 137. *G. coulteri*, Macoun's collection as "*G. crinale*" from Beacon Hill, Victoria, CANA 3474 (was CAN 207), scale bar=50 mm; Fig. 138. *G. robustum* holotype, UC 294572, near Ensenada, Baja California del Norte, Mexico, scale bar=50 mm; Fig. 139. *G. sinicola* holotype, UC 276620, Point Cavallo, Marin Co., California, scale bar=50 mm; Fig. 140. *G. sinicola* holotype (UC 276620), vegetative branch, terete, transverse section, scale bar=30 mm.

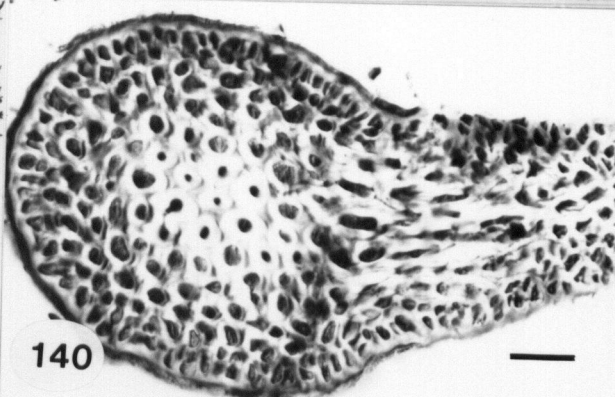
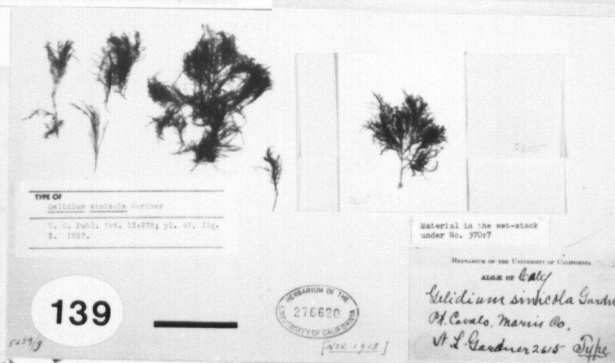
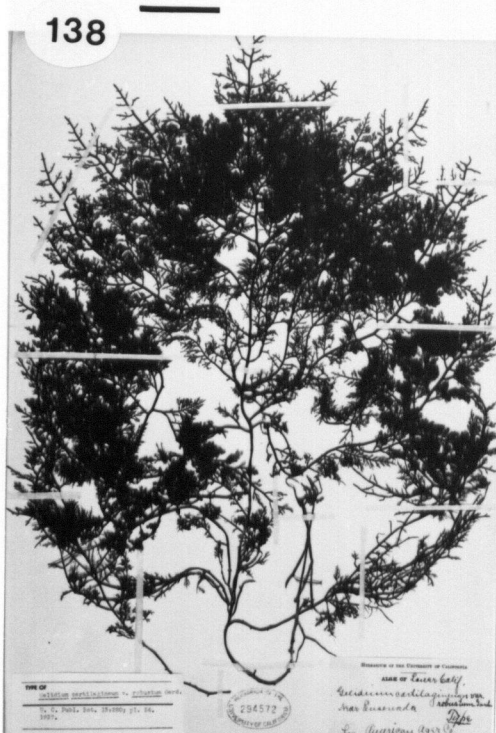
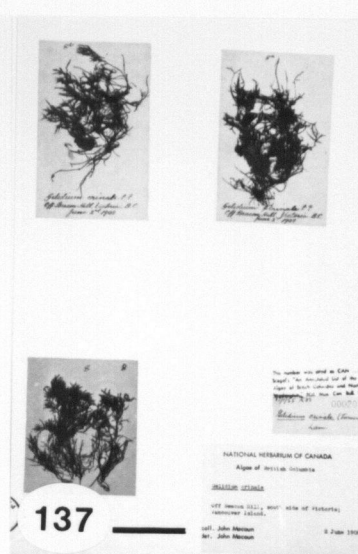
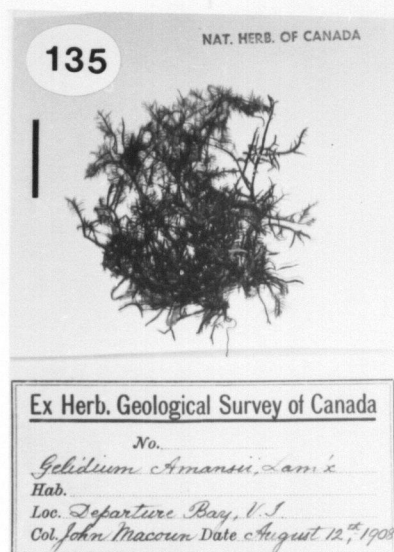
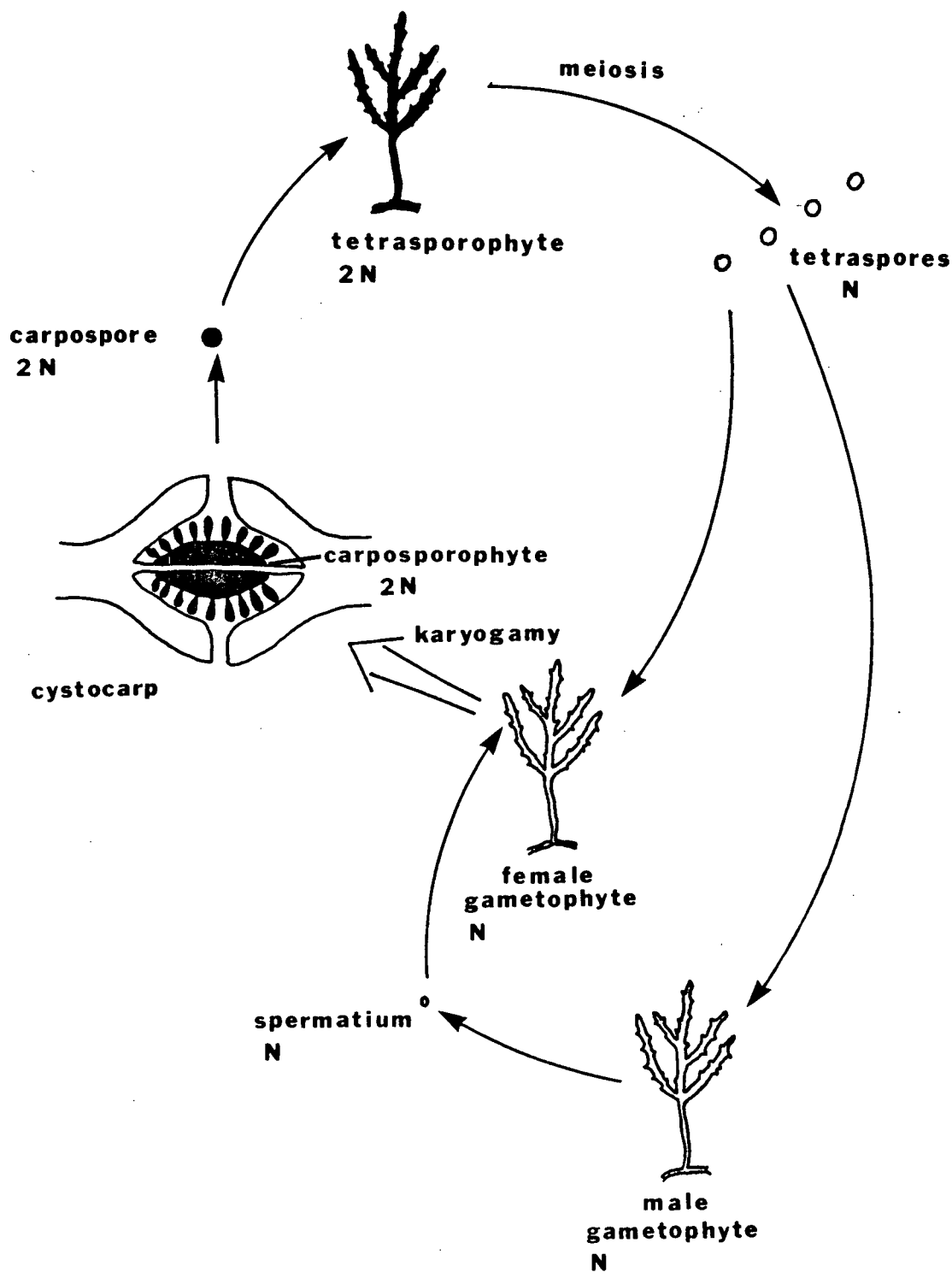
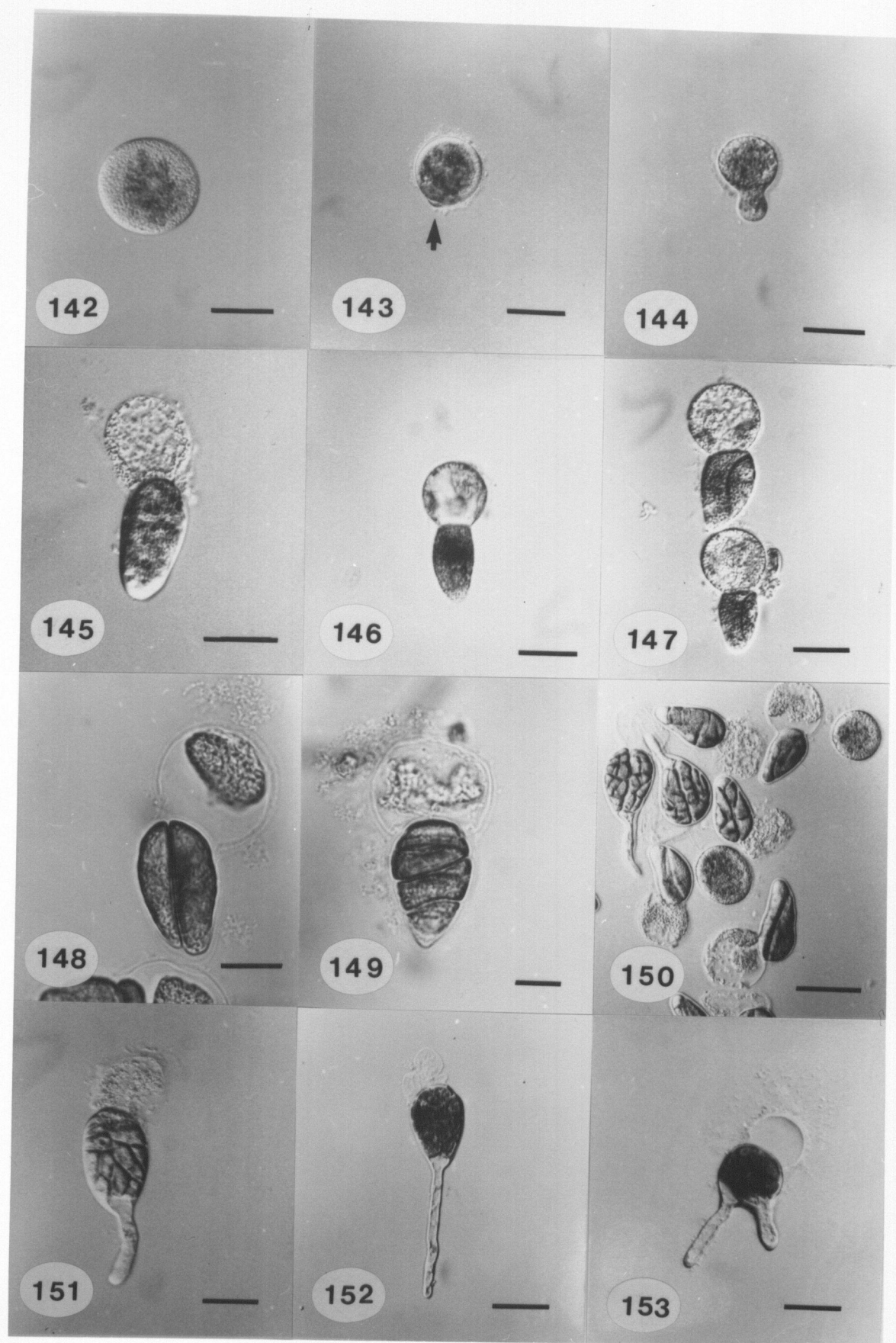


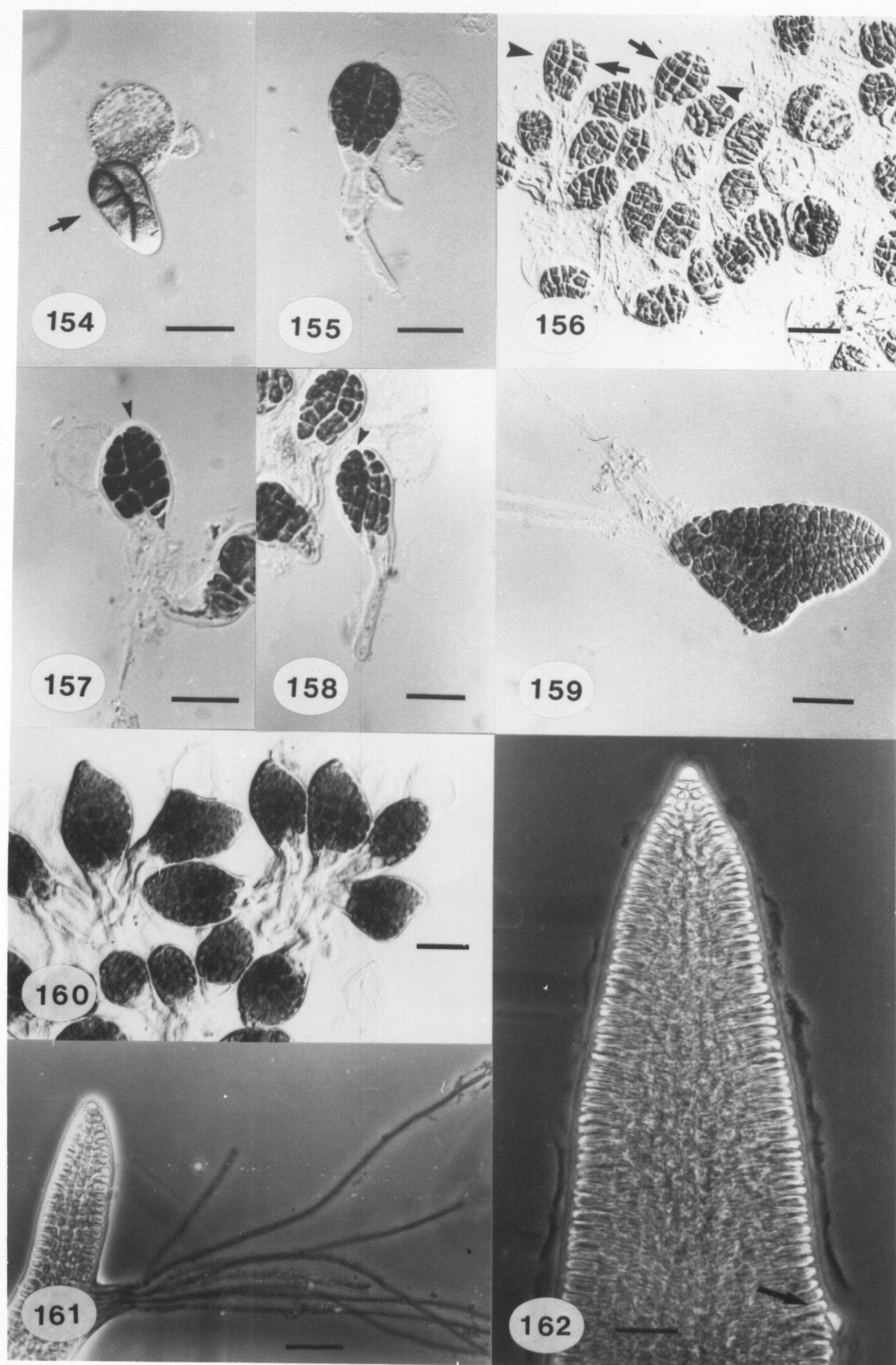
Fig. 141. *Polysiphonia*-type life history. Triphasic (with tetrasporophyte, gametophyte and carposporophyte phases) life history, isomorphic tetrasporophyte and gametophyte, carposporophyte developing in female gametophyte axis, magnified cystocarp shown in longitudinal section; solid (black) structures diploid, hollow (white) structures haploid; not drawn to scale.



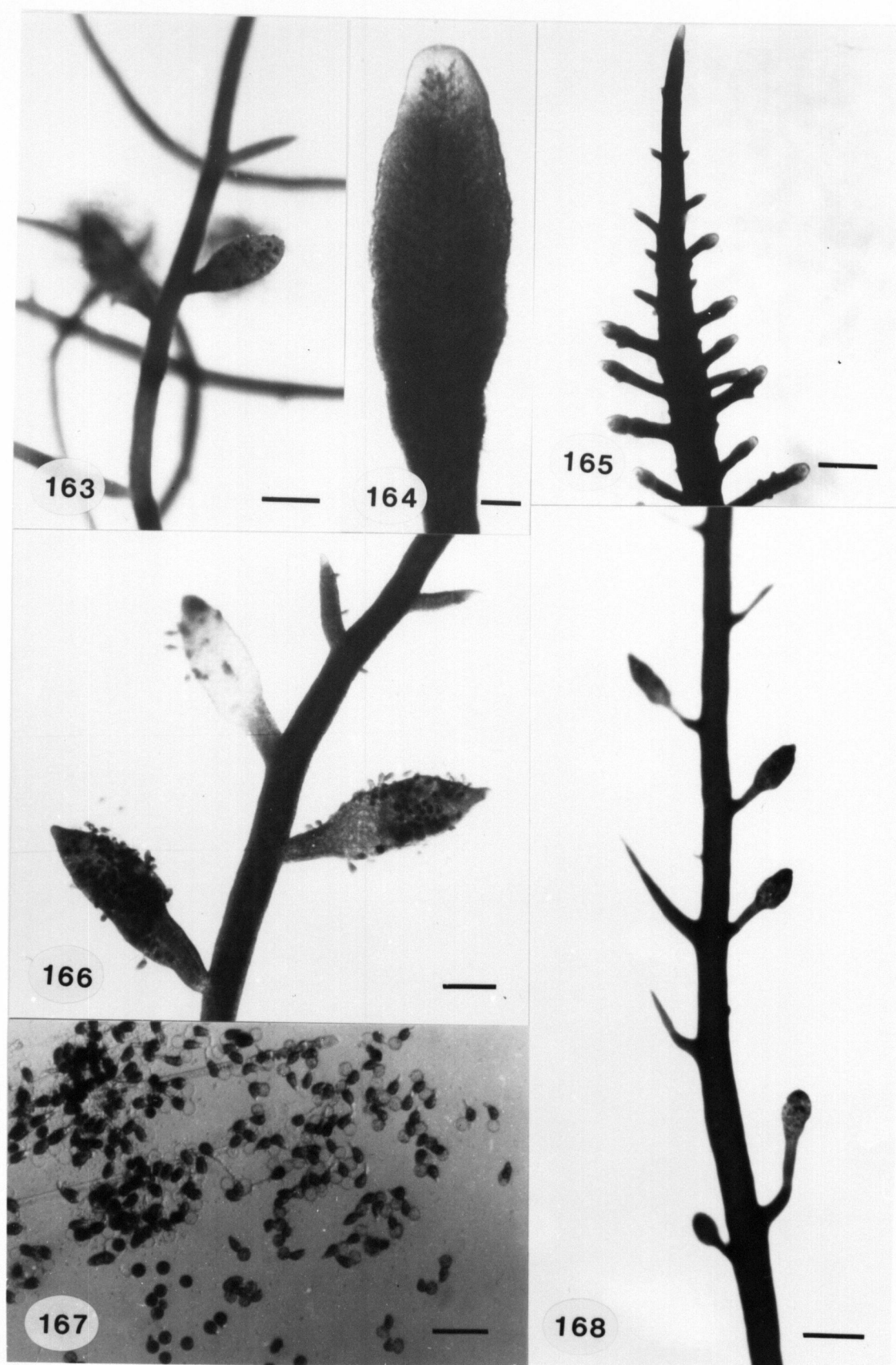
Figs. 142-153. *Gelidium vagum* sporeling development in culture. Fig. 142. Ungerminated tetraspore; Fig. 143. Germ tube (arrow) initiation, gametophyte; Fig. 144. Germ tube enlargement, gametophyte; Figs. 145, 146. Evacuation of cytoplasm from spore into germ tube and wall cutting off germ tube, tetrasporophytes; Fig. 147. First unequal division of germ tube into concave (larger) and fusiform (smaller) cells, tetrasporophytes; Fig. 148. Division of germ tube into concave (right) and fusiform (left) cells, gametophyte; Fig. 149. Transversely divided concave cell, most distal cell of group initiating primary rhizoid, tetrasporophyte; Fig. 150. Variety of developmental stages, original spores all from same cystocarp, tetrasporophytes; Fig. 151. Elongation of primary attachment rhizoid, originated from cell in concave group, tetrasporophyte; Fig. 152. Elongated rhizoid, gametophyte; Fig. 153. Second attachment rhizoid produced (before apical cell initiation), gametophyte. Figs. 142-153 scale bar=30 μ m. Figs. 142, 143 culture source: Tribune Bay, Hornby Is., Figs. 144, 148, 149, 153 culture source: Tribune Bay, Hornby Is., Figs. 145-147, 150, 151 culture source: east side Denman Is., Fig. 152 culture source: Ford Cove, Hornby Is.



Figs. 154-162. *Gelidium vagum* sporeling development in culture. Fig. 154. First division of fusiform cell (arrow), tetrasporophyte; Fig. 155. Fusiform and concave cell groups distinct, tetrasporophyte; Fig. 156. Fusiform (arrowhead) and concave (arrow) cell groups distinct, tetrasporophytes; Fig. 157. Apical cell (arrowhead) formation opposite rhizoid, tetrasporophyte; Fig. 158 Apical cell (arrowhead) formation from fusiform group cell, tetrasporophyte; Fig. 159. Sporeling increased in size, with apical organization, tetrasporophyte; Fig. 160. Gametophyte sporelings with apical organization; Fig. 161. Attachment pad formed from adhesion of elongated cortical cells, tetrasporophyte; Fig. 162. Branch initiation (at arrow), gametophyte. Figs. 154, 155, 157-161 scale bar=30 μm , Figs. 156, 162 scale bar=40 μm . Figs. 154, 155 culture source: east side Denman Is., Figs. 156-159 culture source: Galleon Pt., Hornby Is., Figs. 160-162 culture source: Tribune Bay, Hornby Is.

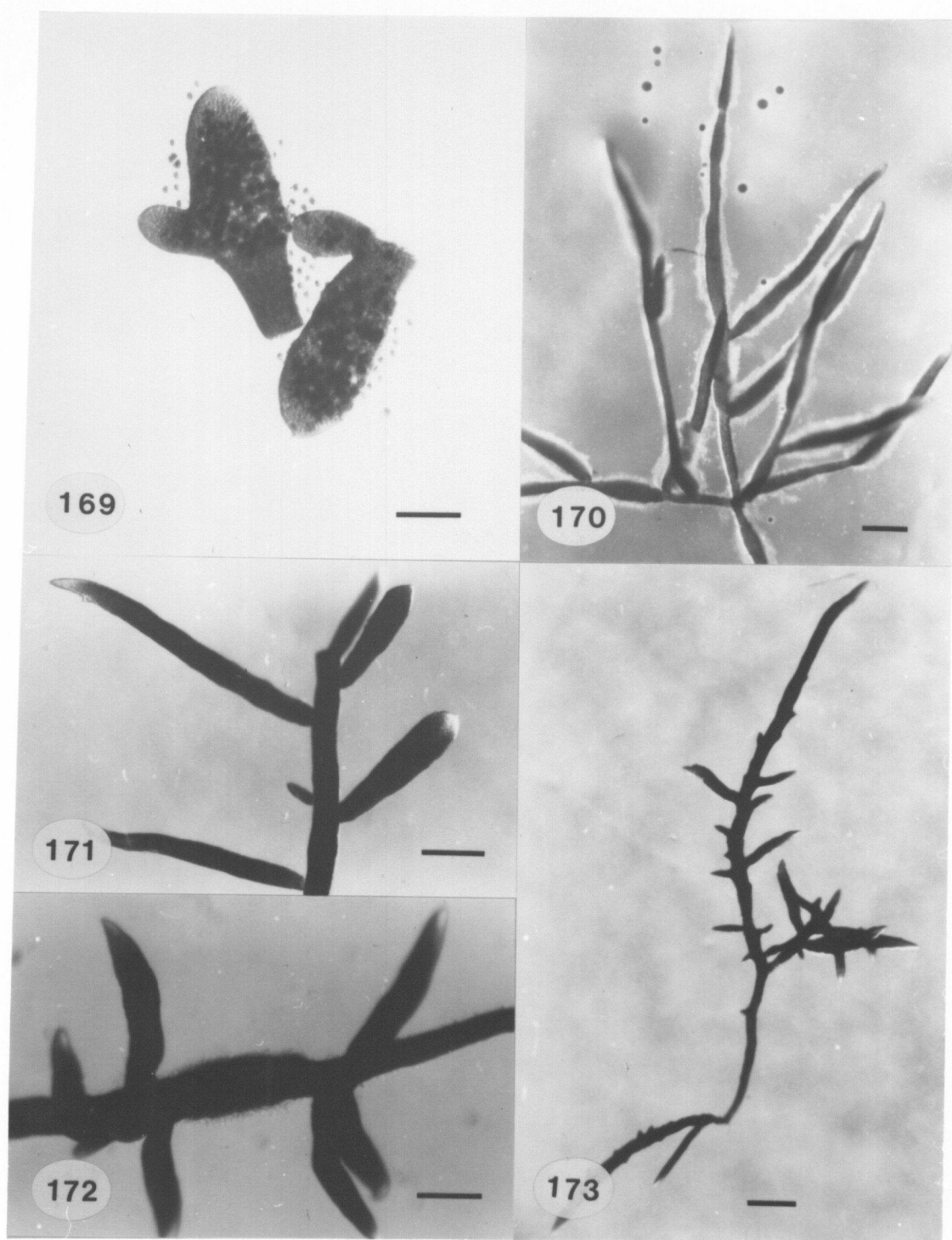


Figs. 163-168. *Gelidium vagum* reproduction in culture. Fig. 163. Tetrasporangial stichidia on tetrasporophyte from carpospores, scale bar=400 μm ; Fig. 164. Gametangial stichidium on gametophyte grown from a tetraspore, scale bar=100 μm ; Fig. 165. Gametangial stichidia on gametophyte from a tetraspore, scale bar=700 μm ; Fig. 166. Tetraspores being released from tetrasporophyte grown from a carpospore, scale bar=500 μm ; Fig. 167. Germination of tetraspores released from tetrasporophyte grown from carpospore, scale bar=300 μm ; Fig. 168. Fertile stichidia on tetrasporophyte grown from carpospore, originally grown from tetraspore, scale bar=500 μm . Figs. 163, 166, 167 culture source: east side Denman Is., Fig. 164, 165 culture source: Galleon Pt., Hornby Is., Fig. 168 culture source: Tribune Bay, Hornby Is.

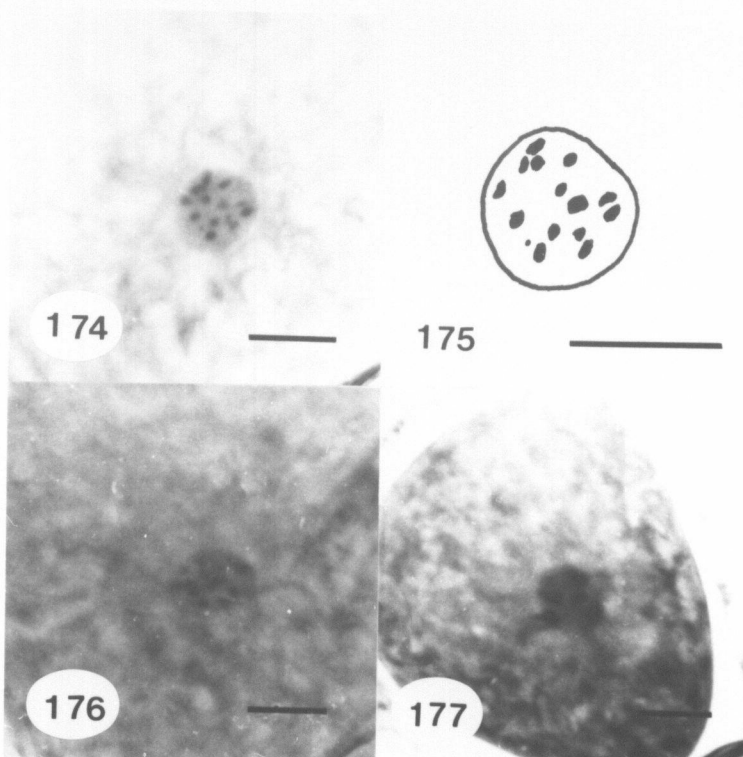


Figs. 169-173. Reproduction of *Gelidium purpurascens* and *G. coulteri* in culture.

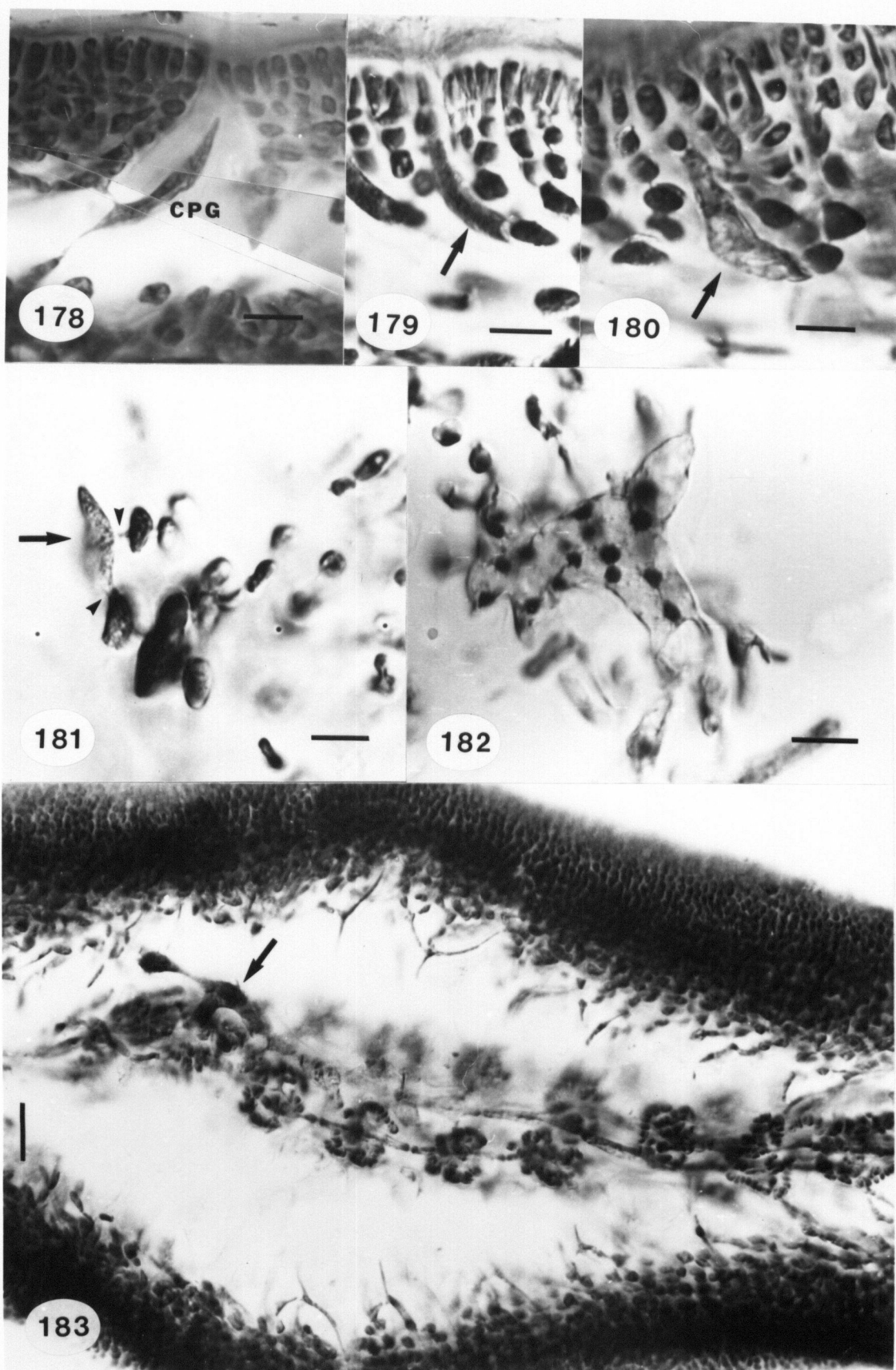
Fig. 169. *Gelidium purpurascens*, tetrasporangial stichidia from field, excised for tetraspore release, scale bar=200 μm ; Fig. 170. *G. purpurascens*, habit of fertile male gametophyte grown from tetraspore, scale bar=500 μm ; Fig. 171. *G. purpurascens*, habit of fertile female gametophyte grown from tetraspore, scale bar=500 μm ; Fig. 172. *G. coulteri*, spermatangial area of male gametophyte grown from tetraspore, scale bar=200 μm ; Fig. 173. *G. coulteri*, habit of fertile male gametophyte grown from tetraspore, scale bar=500 μm . Fig. 174 Oriobar Pt., Gabriola Is., Figs. 170, 171 culture source: Whalebone Bay, Gabriola Is., Figs. 172, 173 culture source: east side Denman Is.



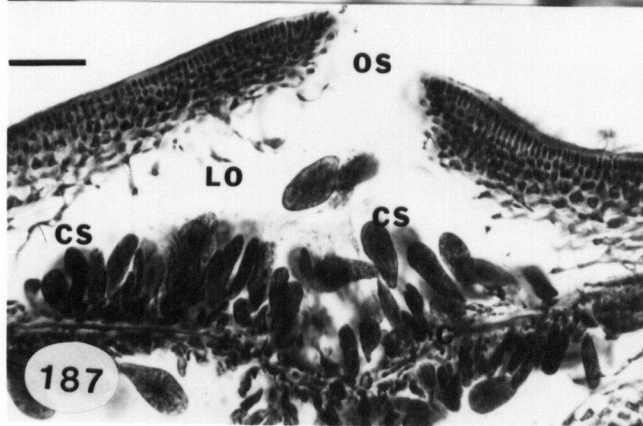
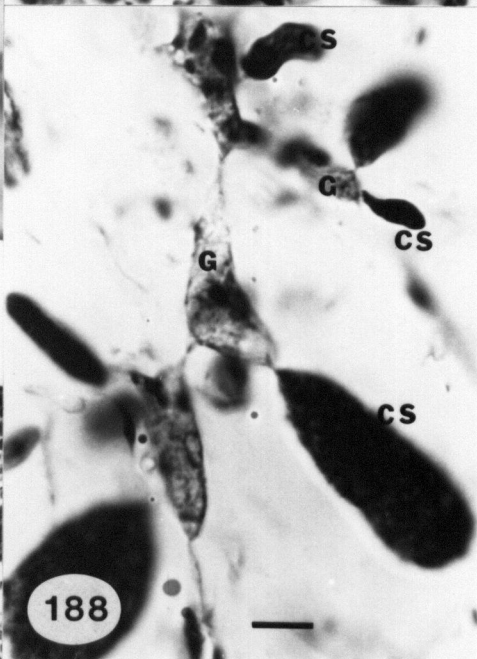
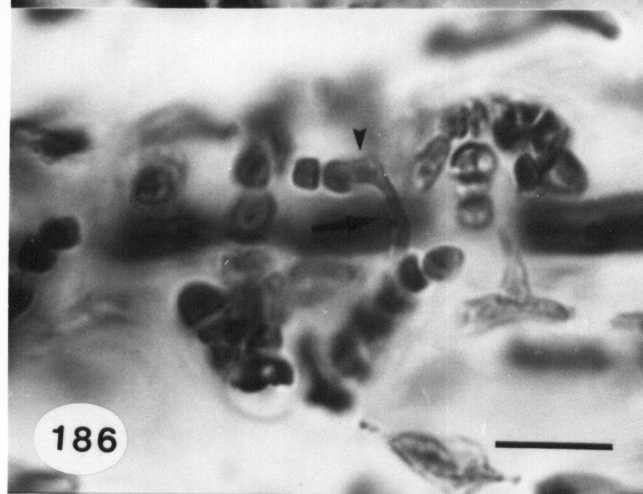
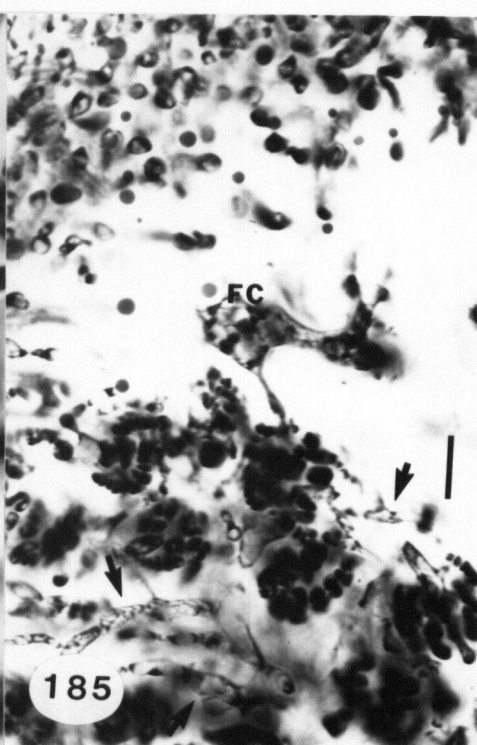
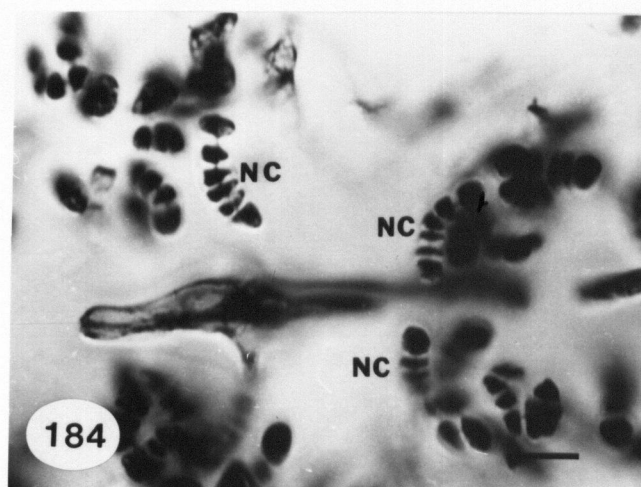
Figs. 174-177. Chromosomes of *Gelidium vagum*. Figs. 174, 176, 177. Prophase I of undivided tetrasporangia, hematoxylin stained, Tribune Bay, Hornby Is.; Figs. 174-176 $n=14-15$, Fig. 175. drawing of nucleus in Fig. 174; Fig. 177. five large chromosomal bodies; scale bar=50 μm for all figures.



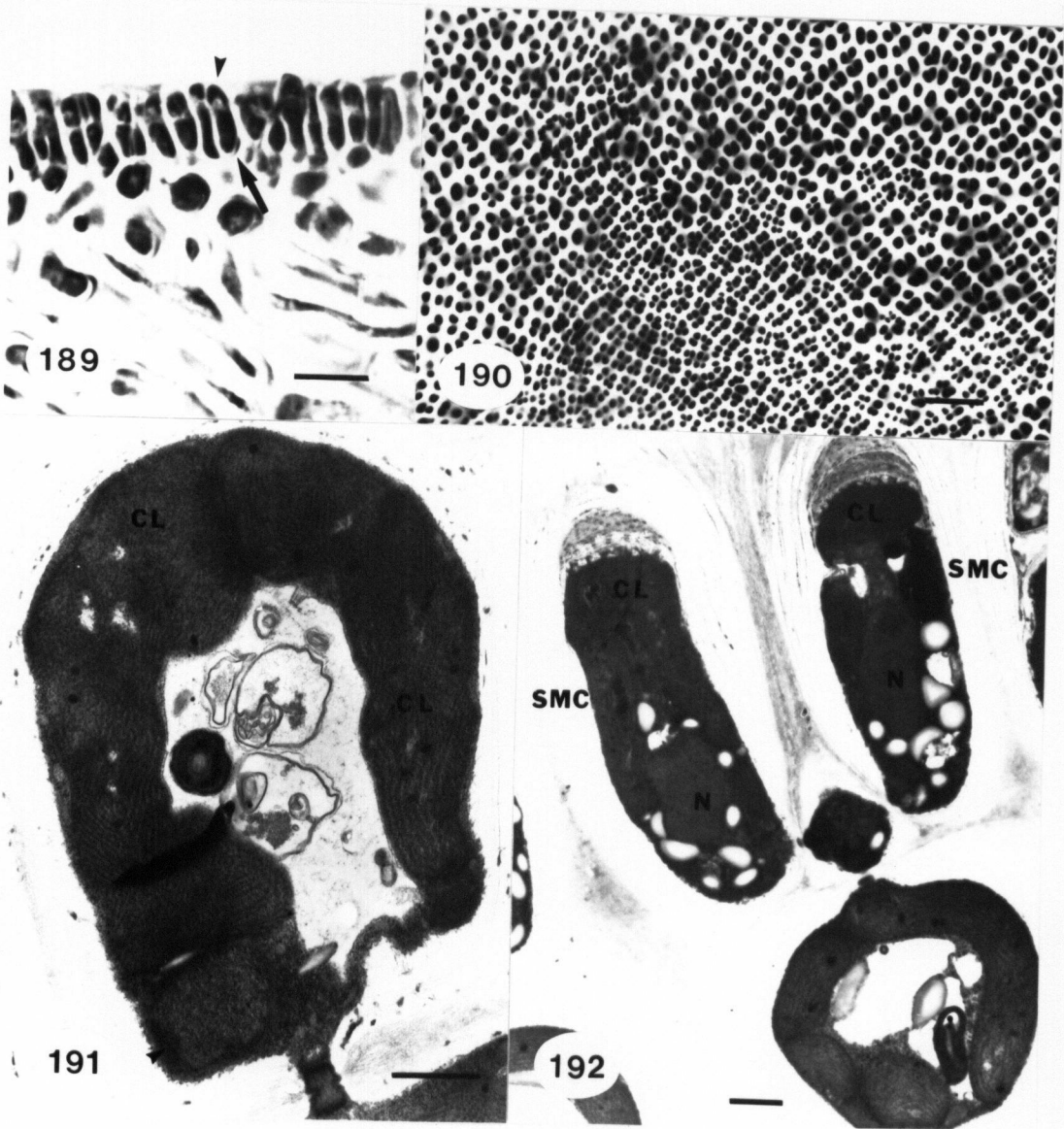
Figs. 178-183. Carpogonium and carposporophyte development in *Gelidium purpurascens* and *G. vagum*. Figs. 178. *Gelidium vagum*, intercalary carpogonium, longitudinal section, scale bar=10 μm ; Figs. 179-183 *G. purpurascens*. Fig. 179. Sessile carpogonium (arrow) with curved basal wall, longitudinal section, scale bar=10 μm ; Fig. 180. Sessile carpogonium (arrow) vacuolate and degenerate, longitudinal section, scale bar=10 μm ; Fig. 181. Fertilized carpogonium (arrow) fusing with cortical cells through two pit connections (arrowheads), showing functional carpogonium is intercalary, squash of longitudinal section, scale bar=10 μm ; Fig. 182. Multilobed, multinucleate fusion cells, squash of longitudinal section, scale bar=10 μm ; Fig. 183. Young carposporophyte, clusters of nutritive chains around second order plate cells and fusion cell (arrow), longitudinal section, scale bar=300 μm . Fig. 178 Ta-no-hama, Iwate-ken, Japan, TNS 25824, Figs. 179, 180, 183 Kirby Pt., Diana Is., Figs. 181, 182 Geer Islets.



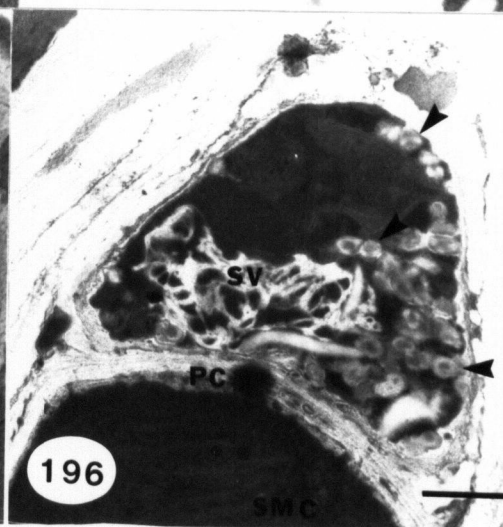
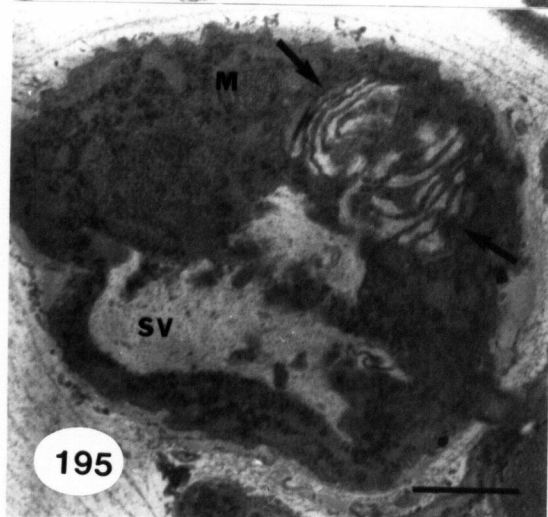
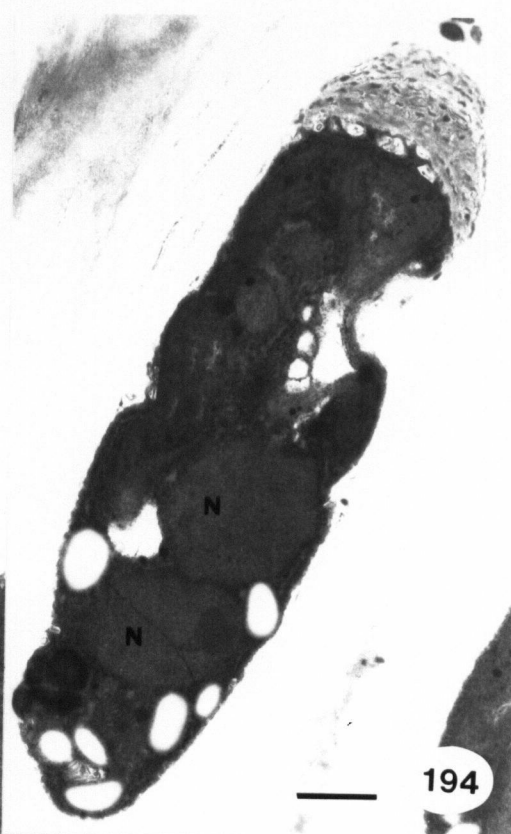
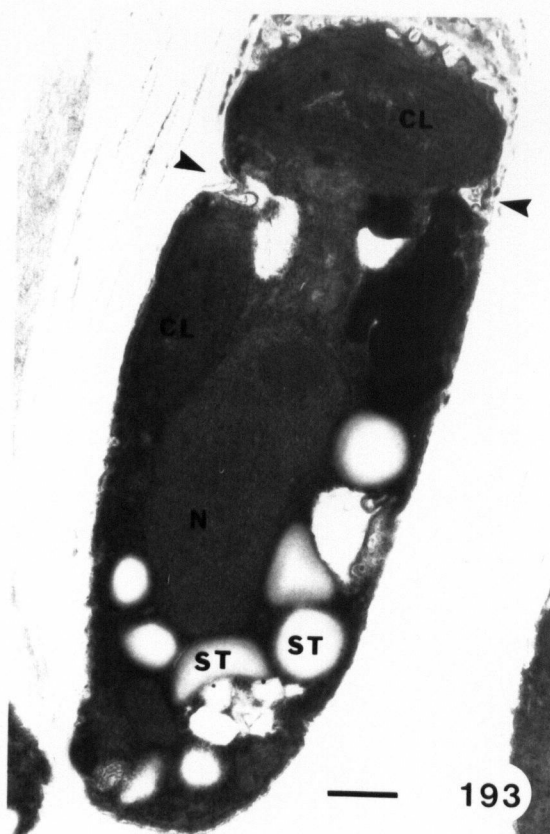
Figs. 184-188. Carposporophyte development in *Gelidium purpurascens* and *G. vagum*. Figs. 184, 185. *Gelidium purpurascens*. Fig. 184. Nutritive chains, longitudinal section, scale bar=10 μm ; Fig. 185. Fusion cell and gonimoblast filaments (arrows), longitudinal section, scale bar=20 μm ; Fig. 186-188. *G. vagum*. Fig. 186. Lobe of gonimoblast cell (arrow) fused with apical cell (arrowhead) of (upper) nutritive chain, longitudinal section, scale bar=10 μm ; Fig. 187. One locule of mature cystocarp showing carposporophyte producing carposporangia, longitudinal sections through ostiole, scale bar=50 μm ; Fig. 188. Gonimoblast cells producing carposporangia terminally and laterally, longitudinal section, scale bar=10 μm . Figs. 184, 185 Geer Islets, Figs. 186, 188 Tribune Bay, Hornby Is., Fig. 187 Ta-no-hama, Iwate-ken, Japan, TNS 25824.



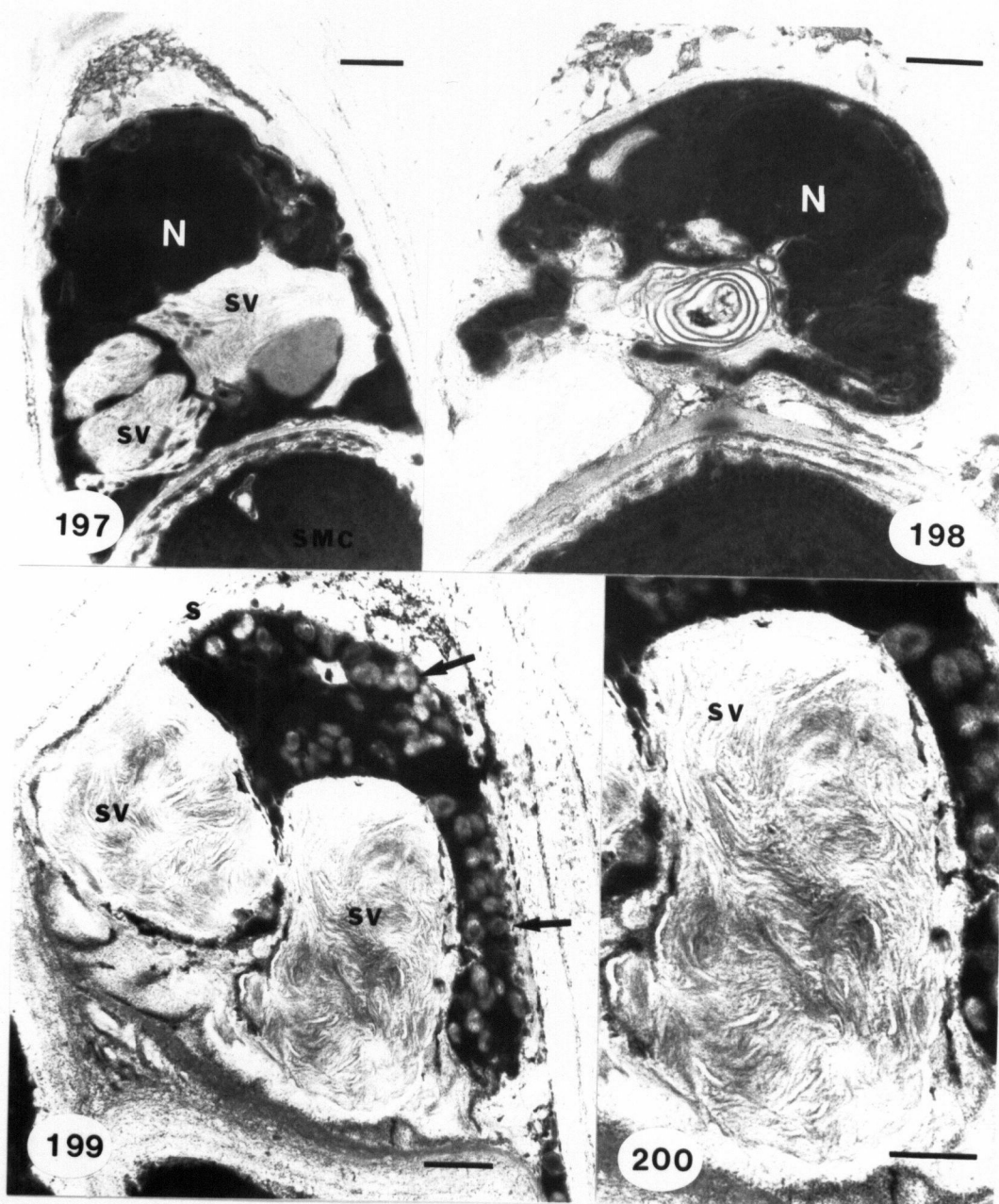
Figs. 189-192. Ultrastructure of spermatogenesis in *Gelidium vagum*. Fig. 189. Spermatangial mother cells (arrow) and spermatangia (arrowhead), longitudinal section, scale bar=10 μm ; Fig. 190. Spermatangia (small cells) and vegetative cortical cells (larger), whole mount, scale bar=30 μm ; Fig. 191. Ultrastructure of vegetative cortical cell with large plastids, basal nucleus (arrowhead) and central vacuole, scale bar=1000 nm; Fig. 192. Ultrastructure of two spermatangial mother cells each with a pit connection to base of a third spermatangial mother cell, which has a pit connection to a cortical cell, scale bar=1000 nm. Figs. 189, 192 Tribune Bay, Hornby Is., Fig. 190 Galleon Pt., Fig. 191 east side Denman Is.



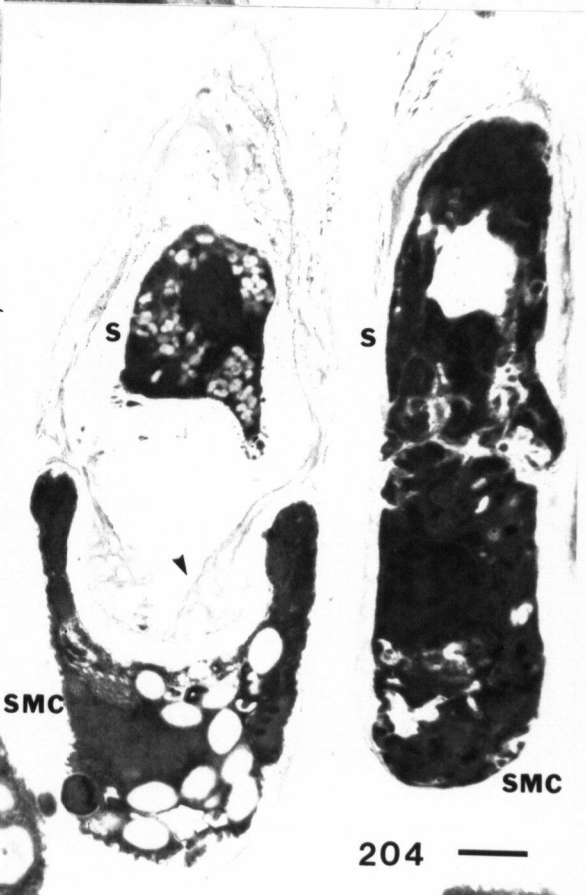
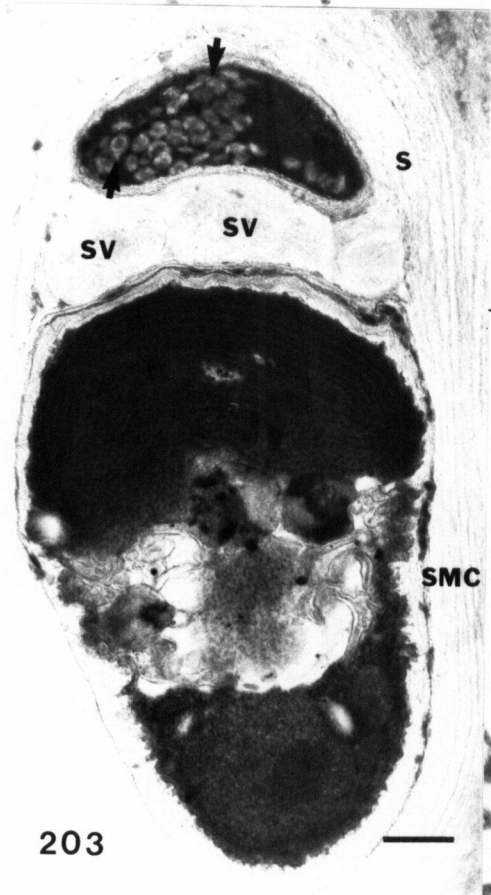
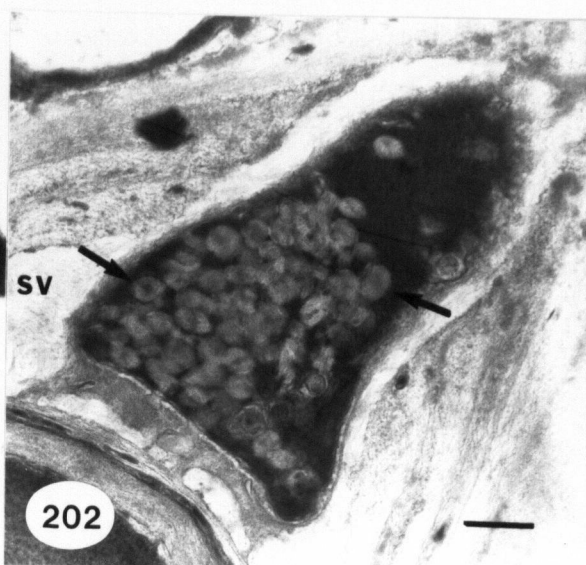
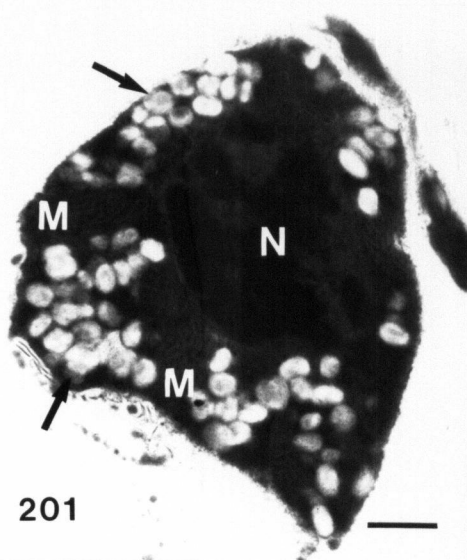
Figs. 193-196. Ultrastructure of spermatogenesis in *Gelidium vagum*. Fig. 193. Spermatangial mother cell with elongate, central nucleus and furrow (arrowheads) possibly excluding apical chloroplast; Fig. 194. Spermatangial mother cell with two nuclei; Fig. 195. Spermatangium with two prominent dictyosomes (arrows) contributing vesicles to young spermatangial vacuole, mitochondrion (M); Fig. 196. Spermatangium with basal pit connection and young spermatangial vacuole, cored vesicles (arrowheads), large nucleus; scale bar=500 nm for all figures. Figs. 193-196 Tribune Bay, Hornby Is.



Figs. 197-200. Ultrastructure of spermatogenesis in *Gelidium purpurascens* and *G. vagum*. Figs. 197, 198. *Gelidium vagum*. Fig. 197. Spermatangium with 2-3 spermatangial vacuoles and large nucleus with condensed chromatin (dark areas), scale bar=300 nm; Fig. 198. Spermatangial vacuole release, nucleus with condensed chromatin (dark areas), scale bar=300 nm; Figs. 199, 200 *G. purpurascens*. Fig. 199. Two spermatangial vacuoles released from spermatangium, spermatangium with many cored vesicles (arrows), scale bar=800 nm; Fig. 200. Spermatangial vacuole, contents whorled fibrillar, scale bar=300 nm. Figs. 197, 198 Tribune Bay, HornbyIs., Figs. 199, 200 cultured Whalebone Bay, Gabriola Is. material.



Figs. 201-204. Ultrastructure of spermatogenesis in *Gelidium purpurascens* and *G. vagum*. Figs. 201, 204. *Gelidium purpurascens*. Fig. 201. Spermatangium with many cored vesicles (arrows), mitochondria (M) and nucleus with condensed chromatin (dark areas), scale bar=40 nm; Figs. 202, 203 *G. vagum*. Fig. 202. Spermatangium starting to release spermatium, spermatangial vacuoles already released, many cored vesicles (arrows) and thick mucilage coating at apex of spermatium, scale bar=400 nm; Fig. 203. Spermatangium with cored vesicles (arrows), spermatangial vacuoles already released, scale bar=500 nm; Fig. 204. Spermatium being released and wall material (arrowhead) secreted by spermatangial mother cell, spermatangial mother cell on right at cytokinesis, scale bar=700 nm. Figs. 201, 204 cultured Whalebone Bay, Gabriola Is. material, Figs. 202, 203 east side Denman Is.



Figs. 205-208. Ultrastructure of spermatogenesis in *Gelidium purpurascens* and *G. vagum*. Figs. 205-207. *Gelidium purpurascens*. Fig. 205. Release of percurrent spermatangia, spermatangial mother cell secreting new wall (arrowheads), note previous wall layer (arrows) surrounding spermatangial mother cell and spermatangium, scale bar=800 nm; Fig. 206. Release of conical spermatium, large nucleus and many cored vesicles (arrows), scale bar=500 nm; Fig. 207. Spermatangial mother cells and old wall layers (arrowheads) of percurrent spermatangia, scale bar=2000 nm; Fig. 208. *G. vagum* spermatangial mother cell with old wall layers and discarded pit plugs (arrowheads) of percurrent spermatangia, scale bar=1000 nm. Figs. 205-207 cultured Whalebone Bay, Gabriola Is. material, Fig. 208 Tribune Bay, Hornby Is.

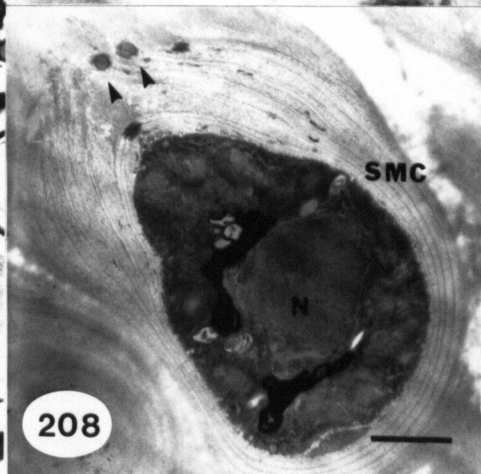
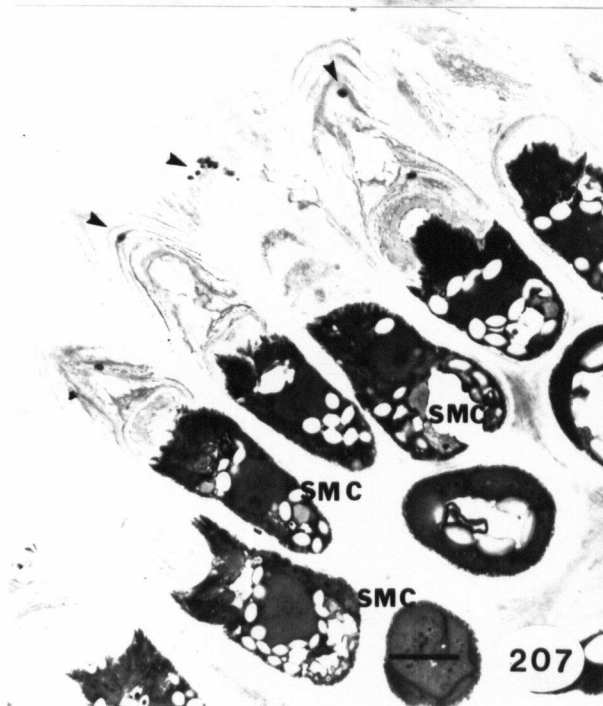
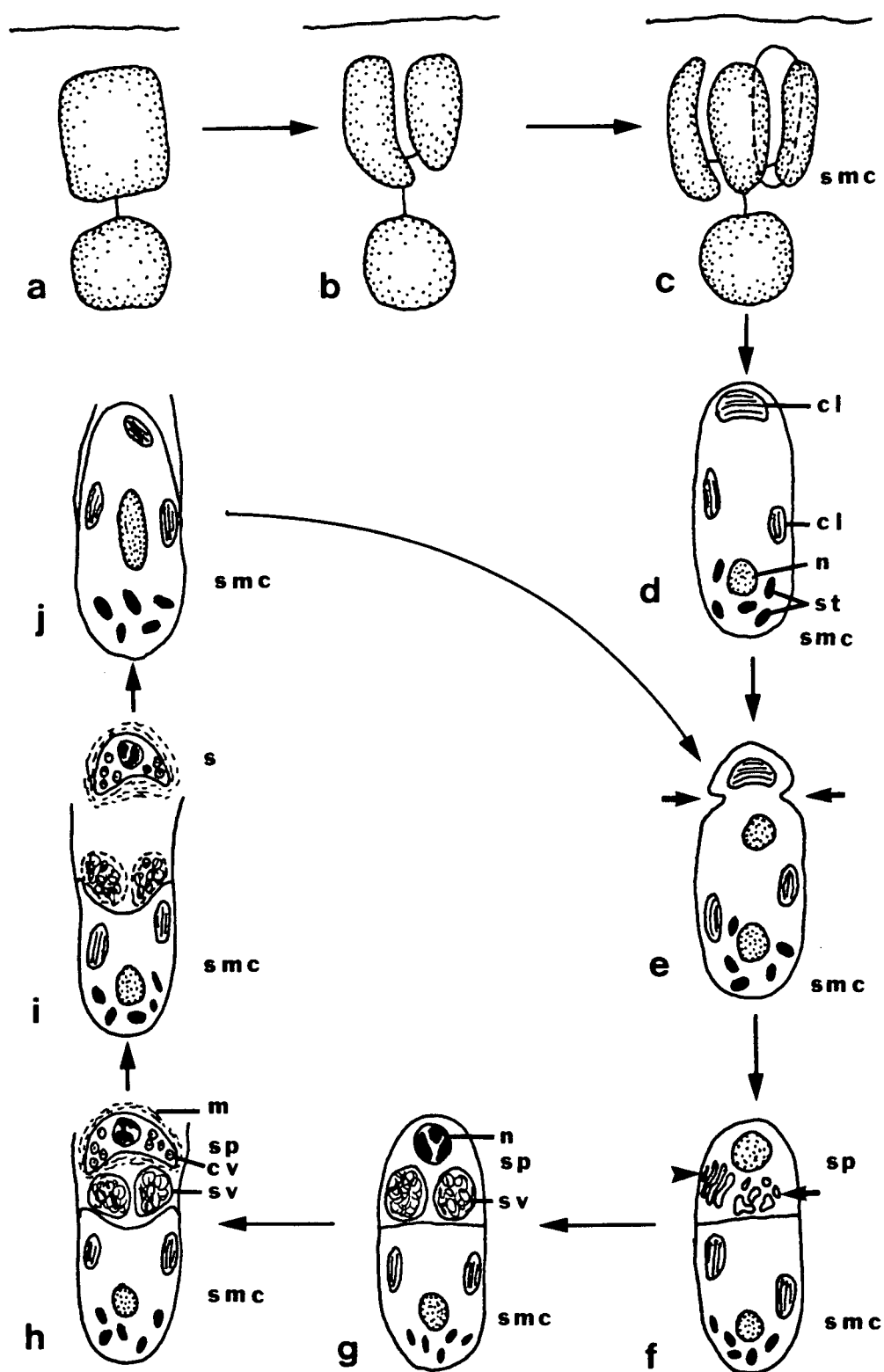
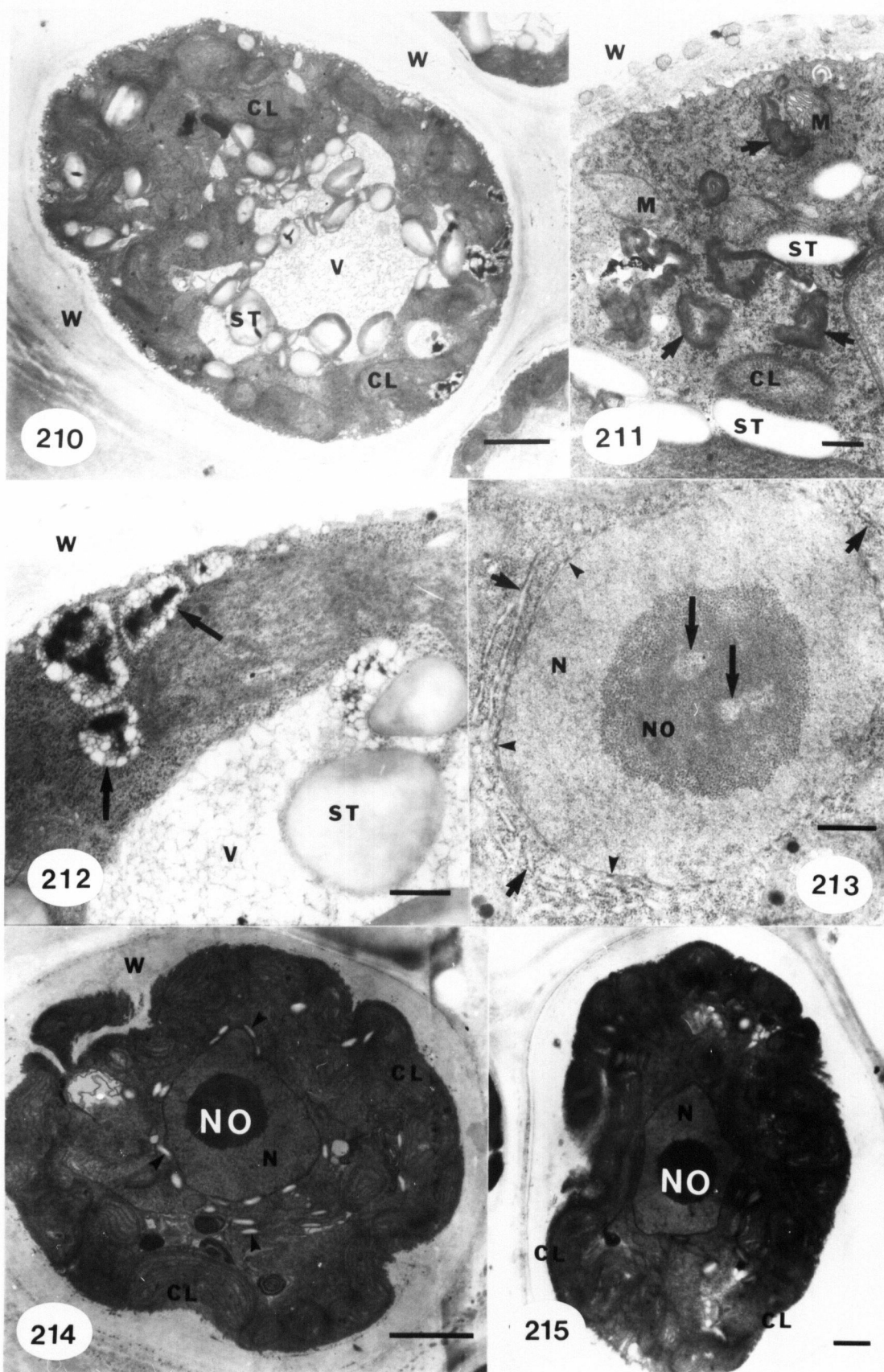


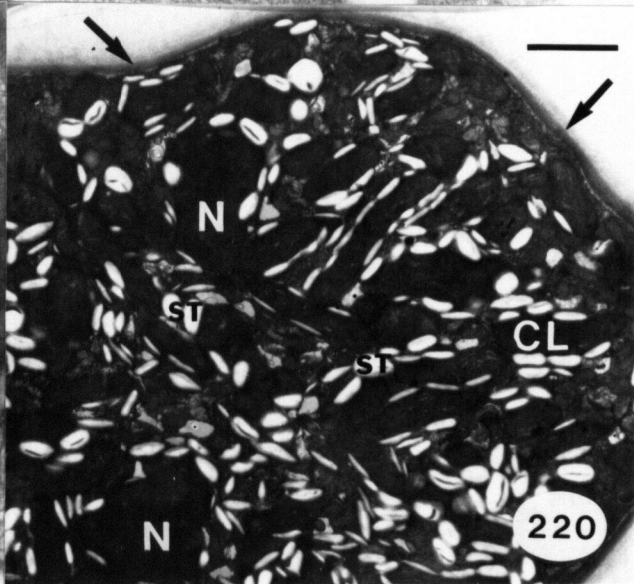
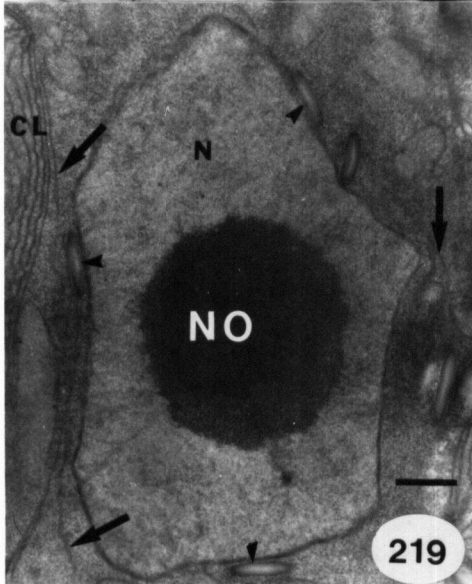
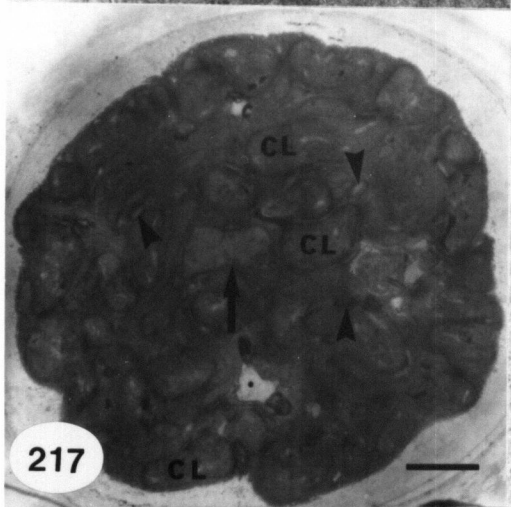
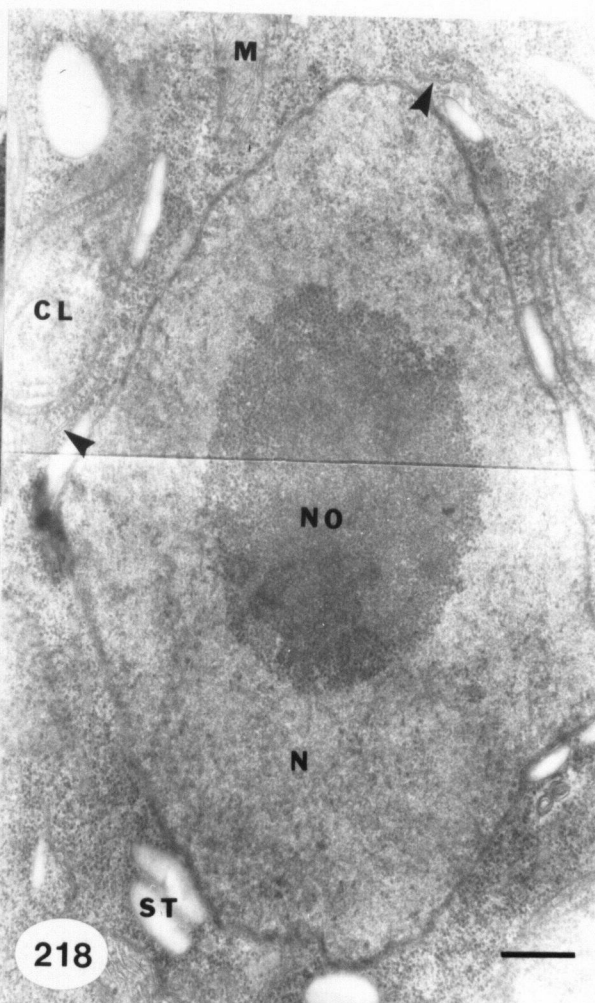
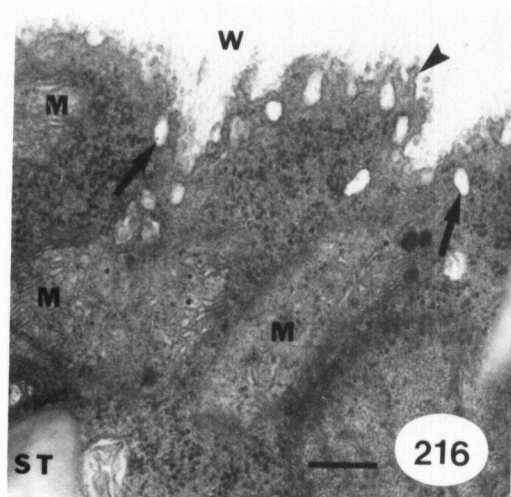
Fig. 209. Summary of spermatogenesis in *Gelidium*. a-c. Longitudinal division of an outer cortical cell to form 4 spermatangial mother cells; d-j. Summary of ultrastructural development of the spermatangium, d. Spermatangial mother cell (smc) with basal nucleus (n), starch grains (st) and chloroplasts (cl) throughout cytoplasm; e. Apical chloroplast (cl) of spermatangial mother cell (smc) being cut off from rest of cell by furrows (arrows); f. Spermatangial mother cell transversely divided to form a spermatangium (sp), spermatangium with a dictyosome (arrowhead) and vesiculated endoplasmic reticulum (arrow); g. Spermatangium (sp) with 2 spermatangial vacuoles (sv) developed from dictyosome-derived vesicles and endoplasmic reticulum, nucleus (n) with condensed chromatin; h. Spermatangial vacuoles (sv) released from the spermatangium, spermatangium with cored vesicles (cv) and an external layer of mucilage-like material (m); i. Spermatium (s) released from spermatangium, spermatium with cored vesicles, a layer of mucilage-like material and nucleus with condensed chromatin; j. Spermatangial mother cell expanded into space vacated by spermatium to form another spermatangium; drawings not to scale.



Figs. 210-215. Ultrastructure of tetrasporogenesis in *Gelidium purpurascens*. Fig. 210. Remains of vacuole (V) in very young tetrasporangium (before expansion of nucleus and thickening of vegetative wall), scale bar=2000 nm; Fig. 211. Membranous material (arrows) extruded from pre-meiotic tetrasporangium, mitochondrion (M), scale bar=400 nm; Fig. 212. Different section of very young tetrasporangium of Fig. 210, material from vacuole (V) incorporated in vesicles (arrows) and released to wall, scale bar=400 nm; Fig. 213. Nucleus of pre-meiotic tetrasporangium with perinuclear endoplasmic reticulum (small arrows), many nuclear pores (arrowheads), large nucleolus, large nucleolar vacuoles (large arrows), scale bar=500 nm; Fig. 214. Pre-meiotic tetrasporangium with central nucleus, large nucleolus, lenticular starch grains (arrowheads) central around the nucleus, scale bar=2000 nm; Fig. 215. Pre-meiotic tetrasporangium, nucleus and nucleolus large, central, chloroplasts mostly peripheral, lenticular starch grains (arrows) around nucleus, scale bar=1000 nm; Figs. 210, 212, 213 Georgina Pt., Mayne Is., Figs. 211, 214, 215 Tribune Bay, Hornby Is. culture.



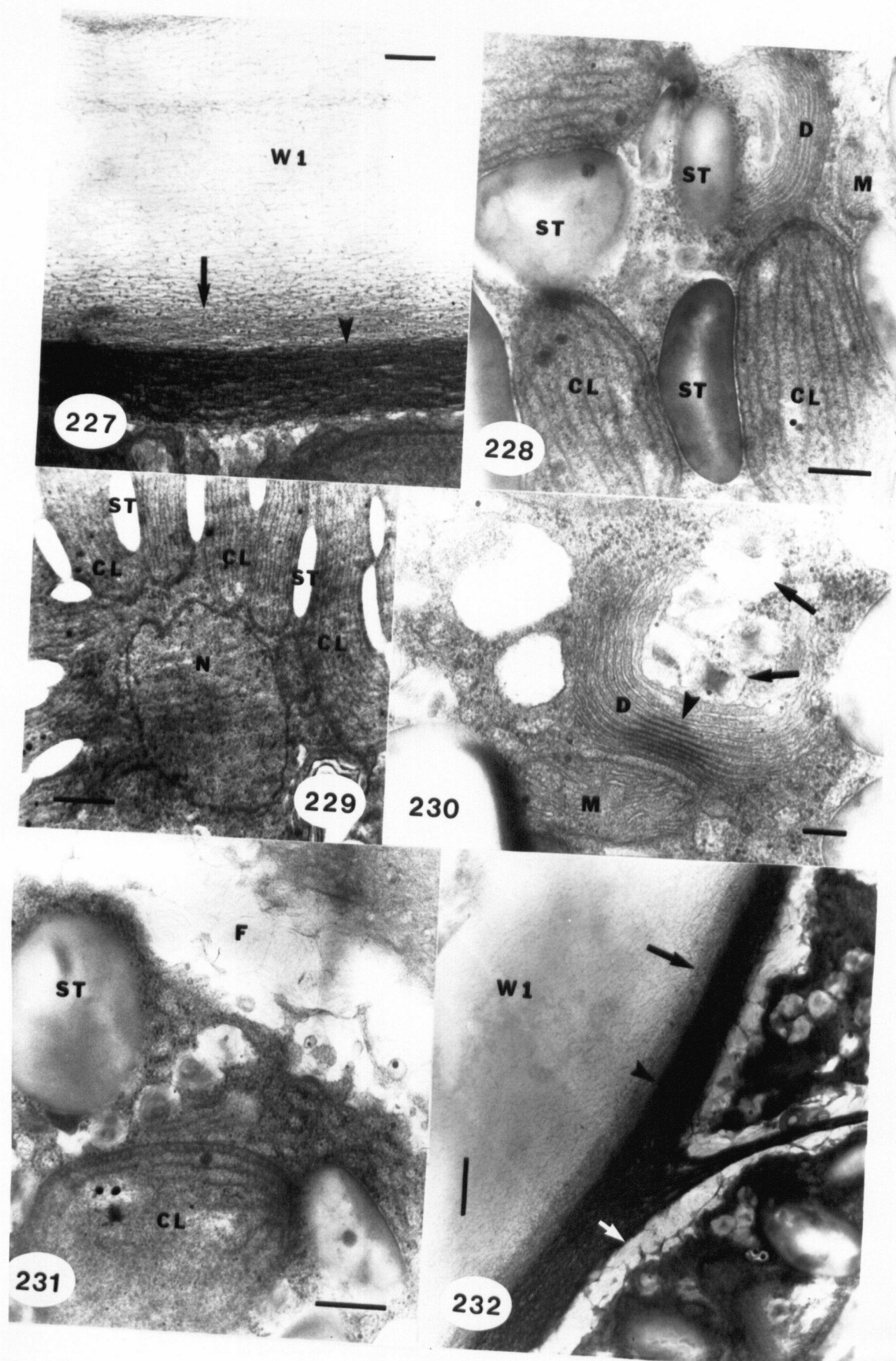
Figs. 216-220. Ultrastructure of tetrasporogenesis in *Gelidium purpurascens*. Fig. 216. Convolut ed plasma membrane (arrowhead) of pre-meiotic tetrasporangium, small transparent vesicles (arrows) of unknown origin release their contents to wall, several mitochondria (M), scale bar=200 nm; Fig. 217. Transverse section of young tetrasporangium, central nucleus (arrow) dumbell shaped, possibly undergoing meiosis, many chloroplasts, lenticular starch grains (arrowheads), scale bar=2000 nm; Fig. 218. Spindle-shaped, single nucleus of pre-meiotic tetrasporangium with large nucleolus, small amount of perinuclear endoplasmic reticulum (arrowheads), lenticular starch grains, mitochondrion (M), scale bar=400 nm; Fig. 219. Elongate nucleus of pre-meiotic tetrasporangium with large nucleolus, perinuclear endoplasmic reticulum (arrows), lenticular starch grains (arrowheads), scale bar=500 nm; Fig. 220. Two nuclei in tetrasporangium after first meiotic division and before cytokinesis, many ovate chloroplasts, many lenticular to ovate starch grains, grey wall layer (layer 2) present (arrow) inside layer 1 wall (W), scale bar=3000 nm; Fig. 216 Georgina Pt., Mayne Is., Figs. 217-220 Tribune Bay, Hornby Is., culture.



Figs. 221-226 Ultrastructure of tetrasporogenesis in *Gelidium purpurascens*. Fig. 221. Flat dictyosome associated with mitochondrion (M) in tetrasporangium about the time of meiosis, dictyosome cisternae contains fibrillar material, scale bar=200 nm; Fig. 222. Vesiculate endoplasmic reticulum at periphery of post-meiotic tetrasporangium, vesicles contain fibrillar material, wall layer 2 present (W2), scale bar=400 nm; Fig. 223. Part of a post-meiotic tetrasporangium with many chloroplasts and starch grains, peripheral dictyosomes flat with many cisternae per stack, mitochondrion (M), scale bar=800 nm; Fig. 224. Tetrasporangium at about the time of karyokinesis, many chloroplasts, many lenticular to ovate starch grains, wall layer 2 (W2) present, scale bar=3000 nm; Fig. 225. Peripheral cytoplasm of post-meiotic tetrasporangium, many dictyosomes and associated mitochondria (M), vesiculate endoplasmic reticulum (arrows), wall layer 2, scale bar=400 nm; Fig. 226. Tetrasporangium at the time of cytokinesis, wall layer 3 (arrow) present interior to layer 2 (arrowhead), many ovate starch grains, many chloroplasts, scale bar=4000 nm; Figs. 221-226 Tribune Bay, Hornby Is., culture.



Figs. 227-232. Ultrastructure of tetrasporogenesis in *Gelidium coulteri* and *G. purpurascens*. Figs. 227, 228, 230-232 *Gelidium purpurascens*. Fig. 227. Tetrasporangial wall layers at cytokinesis, layer 1 (W1), layer 2 (arrow), layer 3 (arrowhead), scale bar=200 nm; Fig. 228. Cytoplasm of tetrasporangium after cytokinesis, starch grains ovate, large dictyosome curved and associated with mitochondrion (M), scale bar=400 nm; Fig. 229. *Gelidium coulteri*, chloroplasts radiating around nucleus, starch grains between chloroplasts, scale bar=1000 nm; Fig. 230. Strongly curved dictyosome associated with mitochondrion (M) in post-cytokinesis tetrasporangium, dark material (arrowhead) between appressed younger cisternae, cored vesicles (arrows) produced at dictyosome maturing face, scale bar=200 nm; Fig. 231. Cored vesicles (arrows) releasing contents to cleavage furrow (F) of tetrasporangium, scale bar=400 nm; Fig. 232. Wall of mature tetrasporangium (after cytokinesis), layer 1 (W1), layer 2 (black arrow), layer 3 (arrowhead), mucilage-like layer (white arrow), scale bar=60 nm; Figs. 227, 228, 230-232 Tribune Bay, Hornby Is., culture, Fig. 229 Brady's Beach, Bamfield.



TABLES

Table 1. Historical summary of genera included in Gelidiaceae (horizontal continuation of table on next page).

Kützing, 1843	Kützing, 1849	J. G. Agardh, 1876	Schmitz, 1889
<i>Acrocarpus</i>	<i>Acrocarpus</i>	<i>Gelidium</i>	<i>Acropeltis</i>
<i>Ctenodus</i>	<i>Chondrodon</i>	<i>Pterocladia</i>	<i>Atractophora</i>
<i>Echinocaulon</i>	<i>Delisea</i>	<i>Ptilophora</i>	<i>Binderella</i>
<i>Gelidium</i>	<i>Echinocaulon</i>	<i>Suhria</i>	<i>Caulacanthus</i>
	<i>Eucterodus</i>	<i>Wurdemannia</i>	<i>Choreocolax</i>
	<i>Gelidium</i>		<i>Gelidium</i>
	<i>Phacelocarpus</i>		<i>Harveyella</i>
	<i>Polycladia</i>		<i>Naccaria</i>
	<i>Thysanocladia</i>		<i>Porphyroglossum</i>
			<i>Pterocladia</i>
			<i>Ptilophora</i>
			<i>Schottmullera</i>
			<i>Suhria</i>
			<i>Wrangelia</i>

Kylin, 1956

Fan, 1961

current, 1988

*Acanthopeltis**Acanthopeltis**Acanthopeltis**Acropeltis**Acropeltis**Beckerella**Beckerella**Beckerella**Gelidiella**Gelidiella**Gelidium**Gelidium**Gelidiocolax**Porphyroglossum**Onikusa**Gelidium**Pterocladia**Porphyroglossum**Porphyroglossum**Ptilophora**Pterocladia**Pterocladia**Suhria**Pterocladiastrum**Ptilophora**Yatabella**Ptilophora**Suhria**Suhria**Yatabella**Yatabella*

Table 2. Sites and dates of field collections of Gelidiales from the study area.

Site	Latitude	Longitude	Dates collected
Brady's Beach, Barkley Sound	48°50'N	125°09'W	24/vi/86
Diplock Island (NE corner), Barkley Sound	48°56'N	125°07'W	28/viii/84
Dixon Island (SE corner), Barkley Sound	48°51'N	125°07'W	27/viii/84
Dodger Channel, Barkley Sound	48°51'N	125°12'W	29/viii/84
Geer Islets, Barkley Sound	48°56'N	125°07'W	28/viii/84
Haines Island, Barkley Sound	48°50'N	125°12'W	29/viii/84
Kirby Point, Diana Island, Barkley Sound	48°51'N	125°13'W	29/viii/84, 27/viii/85, 23/vi/86
Meade Islets, Barkley Sound	48°56'N	125°07'W	28/viii/84
Roquefeuil Bay, Barkley Sound	48°52'N	125°07'W	27/viii/84

Ross Islets, Barkley Sound	48°52'N	125°10'W	28/viii/86
Madrona Point, Orcas Island, Gulf of Georgia	48°41.5'N	122°54'W	24/vi/85
Georgina Point, Mayne Island, Gulf of Georgia	48°52'N	123°17'W	16/xi/85, 14/ii/86, 21/ix/86, 13/viii/87
False Narrows, Gabriola Island, Strait of Georgia	49°08'N	123°49'W	8/x/85
Orlebar Point, Gabriola Island, Strait of Georgia	49°12'N	123°49'W	8/x/85, 11/xii/85, 28/i/86, 3/iii/86, 7/v/86, 4/vi/86, 21/vi/86, 29/viii/86, 28/x/86, 17/ii/87, 5/iii/87, 5/viii/87
Whalebone Bay, Gabriola Island, Strait of Georgia	49°11'N	123°48'W	28/x/86, 27/xi/86, 5/viii/87
Earls Cove, Strait of Georgia	49°45'N	124°00'W	6/iii/85
Finn Cove, Strait of Georgia	49°59'N	124°46'W	6/xi/83

Frolander Bay, Strait of Georgia	49°45'N	124°17'W	5/xi/83
Halfmoon Bay, Strait of Georgia	49°30'N	123°56'W	6/iii/85
Denman Island (E side), Strait of Georgia	49°30.5'N	124°44'W	29/ix/86, 26/viii/87
Ford Cove, Hornby Island, Strait of Georgia	49°30'N		
	124°41'W		1/x/86
Galleon Point, Hornby Island, Strait of Georgia	49°33'N	124°40'W	30/ix/86
Helliwell Park, Hornby Island, Strait of Georgia	49°31'N	124°35'W	23/iv/87
Tribune Bay, Hornby Island, Strait of Georgia	49°32'N	124°38'W	15/x/84, 30/ix/86

Table 3. Herbaria from which loans were obtained and their abbreviations (Holmgren *et al.*, 1981).

Herbarium	Abbreviation
Allan Hancock Foundation, collection housed in LAM	AHFH
National Museum of Canada, Ottawa	CANA
California Academy of Sciences, collection housed in UC	CAS
Friday Harbor Laboratories, Friday Harbor	FHL
Los Angeles County Museum, Los Angeles	LAM
School of Botany, Trinity College, Dublin	TCD
National Science Museum, Tokyo	TNS
University of British Columbia, Vancouver	UBC
University of California, Berkeley	UC
University of Washington, Seattle	WTU

Table 4. Species of Gelidiales recorded from the northeast, northwest and southeast Pacific Ocean areas. Numbered superscripts refer to references as follows:

1. Akatsuka, 1986b
2. Dawson, 1944
3. Dawson, 1953
4. Dawson *et al.*, 1964
5. Santelices, 1977
6. Santelices and Montalva, 1983
7. Santelices and Stewart, 1985
8. Schnetter and Bula Meyer, 1982
9. Stewart, 1976
10. Stewart and Norris, 1981
11. Bangmei *et al.*, 1983

Taxon	Japan	China	Hawaii	Mexico & Calif- ornia	Central and South America
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Acanthopeltis japonica Okamura

●¹

Beckerella irregularis Akatsuka et

●¹

Masaki

Beckerella subcostata (Okamura)

●¹

Kylin

Gelidiella acerosa (Forsskal)

●¹●²●⁸●⁸

Feldmann et Hamel

Gelidiella adnata Dawson

●⁶●⁹

Gelidiella hancockii Dawson

●^{1 1}

Gelidiella ligulata Dawson

●⁴

Gelidiella machristiana Dawson

●⁶

Gelidiella mexicana Dawson

●³

Gelidiella myrioclada Borgesen

●⁶

Gelidiella ramellosa (Kützinger)

●¹

Feldmann et Hamel

Gelidiella refugiensis Dawson

●^{1 1}

Gelidiella stichidiospora Dawson

●⁴

Gelidium amamiense Tanaka

●¹

Gelidium amansii (Lamouroux)

●¹●⁸

Lamouroux

Gelidium arborescens Gardner

●^{1 0}

Gelidium bulae Schnetter

●⁹

Gelidium cartilagineum (L.)

●¹

Greville

Gelidium chilense (Montalva)

●⁸

Santelices et Montalva

<i>Gelidium corneum</i> (Hudson)	● ¹				
Lamouroux					
<i>Gelidium coronadense</i> Dawson				● ⁴	
<i>Gelidium coulteri</i> Harvey				● ⁸	
<i>Gelidium crinale</i> (Turner)	● ¹	● ²	● ⁸		● ⁷
Lamouroux					
<i>Gelidium deciduum</i> Dawson				● ⁴	
<i>Gelidium decumbensum</i> Okamura	● ¹				
<i>Gelidium divaricatum</i> vonMartens	● ¹	● ²			
<i>Gelidium isabelae</i> Taylor	● ¹				● ⁹
<i>Gelidium japonicum</i> (Harvey)		● ⁸			
Okamura					
<i>Gelidium johnstonii</i> Setchell	● ¹			● ⁸	
et Gardner					
<i>Gelidium kintaroi</i> (Okamura)	● ¹				
Yamada					
<i>Gelidium latifolium</i> (Greville)	● ¹				
Bornet					
<i>Gelidium lingulatum</i> Kutzing					● ⁸
<i>Gelidium linoides</i> Kutzing	● ¹				
<i>Gelidium nanum</i> Inagaki	● ¹				
<i>Gelidium nudifrons</i> Gardner				● ⁸	
<i>Gelidium pacificum</i> Okamura		● ⁸			
<i>Gelidium planisculum</i> Okamura		● ⁸			
<i>Gelidium pluma</i> Loomis			● ⁸		

<i>Gelidium polycladum</i> Kutzing	● ¹				
<i>Gelidium polystichum</i> Gardner	● ¹				
<i>Gelidium pristoides</i> Turner	● ¹				
<i>Gelidium pseudointricatum</i>					● ⁷
Skottsberg et Levring					
<i>Gelidium pulchrum</i> Gardner	● ¹				
<i>Gelidium purpurascens</i> Gardner	● ¹			● ⁸	
<i>Gelidium pusillum</i> (Stackhouse)	● ¹	● ⁸	● ⁸	● ⁸	● ⁷
LeJolis					
<i>Gelidium reediae</i> Loomis			● ⁸		
<i>Gelidium rex</i> Santelices et Abbott					● ⁸
<i>Gelidium rigens</i> Greville	● ¹				
<i>Gelidium robustum</i> (Gardner)				● ⁸	
Hollenberg et Abbott					
<i>Gelidium sclerophyllum</i> Taylor				● ⁴	● ⁹
<i>Gelidium subfastigiatum</i> Okamura	● ¹				
<i>Gelidium sinicola</i> Gardner				● ⁷	
<i>Gelidium vagum</i> Okamura	● ¹	● ²			
<i>Gelidium yamadae</i> (Okamura) Fan	● ¹				
<i>Onikusa japonica</i> (Harvey)	● ¹				
Akatsuka					
<i>Pterocladia bulbosa</i> Loomis				● ⁸	
<i>Pterocladia caerulescens</i> (Kutzing)				● ⁸	
Santelices					

Pterocladia caloglossoides (Howe)

● 6

● 10

● 5

Dawson

Pterocladia capillacea (Gmelin)

● 1

● 8

● 8

● 8

● 7

Bornet et Thuret

Pterocladia densa Okamura

● 1

Pterocladia mcnabbiana Dawson

● 10

● 5

Pterocladia media Dawson

● 11

● 7

Pterocladia nana Okamura

● 1

Pterocladia tenuis Okamura

● 1

● 2

Yatabella hirsuta Okamura

● 1

Table 5. Differences between *Gelidium purpurascens* and *Gelidium robustum*

Character	<i>Gelidium purpurascens</i>	<i>Gelidium robustum</i>
height	usually not >15 cm	commonly >15 cm
main axis	1 - several	1
branching	dense, branches close together, separated by 1-2 mm	coarse, loose, branches separated by at least 3-5 mm
higher order branch bases	not constricted	constricted
inner cortex	cells unordered	cells ordered in pallisade-like rows
tetrasporangial stichidium	narrow sterile margin	wide sterile margin

Table 6. Anatomical differences between *Gelidium johnstonii* and *Gelidium vagum*

Characters	<i>Gelidium johnstonii</i>	<i>Gelidium vagum</i>
vegetative: outer cortical cells	in anticlinal files	not in anticlinal files
tetrasporangial stichidia:		
shape	spatulate	ovate, tapered apically
apex	blunt	pointed
sterile margin	present	absent
apical cell	even or recessed	protruding
apical furrow	present	absent
flattened	yes	no - at most compressed
cystocarp: third order filaments	intact, stretched	not intact, broken

APPENDIX 1. HERBARIUM SPECIMENS EXAMINED

F = female gametophyte, G = gametophyte, M = male gametophyte,
T = tetrasporophyte, specimens without a letter designation are vegetative

Gelidium coulteri

AHFH 53929	California	ISOTYPE
CANA 3474	Beacon Hill, Victoria	
FHL 3055	Cape Alava, Washington	
TCD	California	photograph of LECTOTYPE
TCD	California	ISOTYPE
UBC A906	Monterey, California	T
UBC A907	Monterey, California	
UBC A1449	Moss Beach, California	
UBC A2793	Amos Is., Kyuquot, Vancouver Is.	
UBC A4890	Mukkaw Bay, Washington	
UBC A10812	Mills Peninsula, Vancouver Is.	
UBC A10814	Esteban Point, Vancouver Is.	
UBC A11940	McLean Is., Vancouver Is.	
UBC A11947	Spring Is., Kyuquot, Vancouver Is.	
UBC A12073	Miracle Beach, Vancouver Is.	
UBC A12294	Spring Is., Kyuquot, Vancouver Is.	

UBC A12298	McLean Is., Vancouver Is.	
UBC A12299	McLean Is., Vancouver Is.	
UBC A12300	McLean Is., Vancouver Is.	
UBC A13259	Perez Rocks, Vancouver Is.	
UBC A13478	Walters Cove, Vancouver Is.	
UBC A13658	La Jolla, California	
UBC A14403	Mukkaw Bay, Washington	T
UBC A15173	Malaspina Narrows, Queen Charlotte Strait	
UBC A19739	Brooks Peninsula, Vancouver Is.	
UBC A24527	Pescadero Point, San Mateo Co., California	
UBC A24837	Mukkaw Bay, Washington	
UBC A28351	Orlebar Point, Gabriola Is., B.C.	
UBC A31363	Decanso Bay, Gabriola Is., B.C.	
UBC A31426	Davison's Beach, Gabriola Is., B.C.	
UBC A36197	Brooks Peninsula, Vancouver Is., B.C.	
UBC A36198	False Narrows, Gabriola Is., B.C.	
UBC A36199	Lock Bay, Gabriola Is., B.C.	
UBC A36372	Jackobson Point, Brooks Peninsula, Vancouver Is., B.C.	
UBC A36373	Bunsby Is., Vancouver Is., B.C.	
UBC A36374	Bunsby Is., Vancouver Is., B.C.	
UBC A36545	Amos Is., Vancouver Is., B.C.	
UBC A36711	Bunsby Is., Vancouver Is., B.C.	
UBC A36712	Bunsby Island, Vancouver Is., B.C.	
UBC A36713	Bunsby Is., Vancouver Is., B.C.	

UBC A37077	Fossil Beach, Grassy Is., B.C.	
UBC A37078	Fossil Beach, Grassy Is., B.C.	
UBC A37079	Grassy Is., B.C.	
UBC A37219	Yellow Bluff, Vancouver Is., B.C.	
UBC A37220	Yellow Bluff, Vancouver Is., B.C.	
UBC A37221	Fossil Beach, Grassy Is., B.C.	T
UBC A37223	Yellow Bluff, Vancouver Is., B.C.	
UBC A37402	Pincer Is., Vancouver Is., B.C.	T
UBC A37403	Pincer Is., Vancouver Is., B.C.	T
UBC A37404	Nootka Is., Vancouver Is., B.C.	T
UBC A38472	Lawn Point, Vancouver Is., B.C.	
UBC A38473	Lawn Point, Vancouver Is., B.C.	
UBC A38474	Lawn Point, Vancouver Is., B.C.	
UBC A39128	Arab Cove, Vancouver Is., B.C.	
UBC A40404	Mukkaw Bay, Washington	
UBC A40405	Mukkaw Bay, Washington	
UBC A40415	Crescent Beach, Clallam Co., Washington	T
UBC A40467	Diana Is., Barkley Sound, B.C.	
UBC A40468	Diana Is., Barkley Sound, B.C.	
UBC A40469	Diana Is., Barkley Sound, B.C.	
UBC A40470	Diana Is., Barkley Sound, B.C.	
UBC A42814	Lawn Point, Vancouver Is., B.C.	
UBC A43519	Fleming Is., Barkley Sound, B.C.	
UBC A43520	Reeks Is., Barkley Sound, B.C.	
UBC A43521	Aguilar House Beach, Bamfield, B.C.	

UBC A43522	Aguilar House Beach, Bamfield, B.C.	
UBC A43523	Effingham Is., Barkley Sound, B.C.	
UBC A47159	S. California (L.A. Co.)	
UBC A50505	Mussel Point, Monterey, California	
UBC A53814	Kirby Point, Diana Is., Barkley Sound, B.C.	T
UBC A53975	Brooks Peninsula, Vancouver Is., B.C.	
UBC A60236	Aguilar House Beach, Bamfield, B.C.	
UBC A60254	Diana Is., Barkley Sound, B.C.	
UBC A60268	Execution Rock, Barkley Sound, B.C.	
UBC A60460	Aguilar House Beach, Bamfield, B.C.	
UBC A61984	Pigeon Point, California	T
UBC A62016	Montana de Oro, California	T
UBC A64934	Ladysmith, B.C.	
UC 305332	Moss Beach, Pacific Grove, California	
WTU 246345	Steamboat Is., Thurston Co., Washington	
WTU 246351	Mukkaw Bay, Washington	

Gelidium johnstonii

AHFH 69	San Francisquito Bay, Baja California, Mexico	T, ISOTYPE
AHFH 2211	Pond Is., off Angel de la Guardia Is., Mexico	G
AHFH 4150	Ensenada Bocochibampo, near Guayamas, Mexico	T

AHFH 4156	Bahia Carrizal, near Cabo Circo, Sonora, Mexico	T
AHFH 4179	Ensenada Bocochoibampo, Sonora, Mexico	G
AHFH 4192	Isla Jorge, Gulf of California, Mexico	
AHFH 4193	Isla Patos, near Isla Tiburon, Gulf of California, Mexico	T
AHFH 4194	Isla Patos, near Isla Tiburon, Gulf of California, Mexico	T
AHFH 50267	Bahia Aqua Verde, Baja California, Mexico	G,T
AHFH 50268	Punta Perico, Salinas Bay, Carmen Is., Gulf of California, Mexico	G,T
AHFH 50299	Puerto Escondido, Baja California, Mexico	G,T
CAS 1343 in UC	San Francisquito Bay, Baja California, Mexico	G, T HOLO- TYPE
CAS 484385 in UC	San Francisquito Bay, Baja California, Mexico	T, ISOTYPE
CAS 484386 in UC	San Francisquito Bay, Baja California, Mexico	T, ISOTYPE
CAS 464388 in UC	San Francisquito Bay, Baja California, Mexico	G, T ISO- TYPE
CAS 484390 in UC	San Marcos Is., Gulf of California, Mexico	T, PARA- TYPE
LAM 52684	Puerto Escondido, Gulf of California, Mexico	T
LAM 52894	SW end of Isla Partida, Gulf of California, Mexico	T

Gelidium purpurascens

CANA 3473	Beacon Hill, Victoria, B.C.	T
CANA 3740	Beacon Hill, Victoria, B.C.	
CANA 3843	Departure Bay, Vancouver Is., B.C.	T
CANA 4346	Departure Bay, Vancouver Is., B.C.	
CANA 4349	Departure Bay, Vancouver Is., B.C.	
FHL 1205	Santa Cruz, California	T
FHL 2849	Mitchell Point, San Juan Is., Washington	
UBC A903	Ensenada, California	T
UBC A1799	East Sound, Orcas Is., Washington	
UBC A1952	Ucluelet, Vancouver Is., B.C.	T
UBC A4263	Garden Is., Kyuquot, B.C.	T
UBC A4264	Garden Is., Kyuquot, B.C.	T
UBC A4265	Kains Is., B.C.	
UBC A4402	American Camp Beach, San Juan Is., Washington	T
UBC A4403	American Camp Beach, San Juan Is., Washington	T
UBC A5195	Minnesota Seaside Station, Vancouver Is., B.C.	
UBC A6744	Sunset Bay, Oregon	
UBC A6755	Cape Arago, Oregon	T
UBC A10310	Cortes Is., B.C.	
UBC A10514	Catala, Vancouver Is., B.C.	T

UBC A10813	Esteban Point, Vancouver Is., B.C.	M
UBC A11271	Ogden Point Breakwater, Victoria, B.C.	
UBC A11278	Rosebush Is., Vancouver Is., B.C.	T
UBC A11329	Dorcas Point, Strait of Georgia, B.C.	T
UBC A11929	McLean Is., Vancouver Is., B.C.	T
UBC A11937	McLean Is., Vancouver Is., B.C.	T
UBC A11988	Spring Is., Vancouver Is., B.C.	T
UBC A12296	McLean Is., Vancouver Is., B.C.	T
UBC A13479	Walters Cove, Vancouver Is., B.C.	T
UBC A17771	Acous Peninsula, B.C.	T
UBC A19032	Hisnit Is., B.C.	
UBC A28349	Orlebar Point, Gabriola Is., B.C.	F
UBC A28646	Twin Beaches, Gabriola Is., B.C.	
UBC A31414	Lock Bay, Gabriola Is., B.C.	T
UBC A35158	Arab Cove, Vancouver Is., B.C.	T
UBC A35175	Arab Cove, Vancouver Is., B.C.	
UBC A36371	Brooks Peninsula, Vancouver Is., B.C.	T
UBC A36710	Bunsby Is., Vancouver Is., B.C.	T
UBC A36875	Union Is., Vancouver Is., B.C.	T
UBC A36876	Amos Is., Vancouver Is., B.C.	F
UBC A37603	Blight Is., Vancouver Is., B.C.	T
UBC A38471	Lippy Point, Vancouver Is., B.C.	T
UBC A38475	Arab Cove, Vancouver Is., B.C.	T
UBC A38734	Arab Cove, Vancouver Is., B.C.	T
UBC A38908	Arab Cove, Vancouver Is., B.C.	T

UBC A39126	Lawn Point, Vancouver Is., B.C.	F
UBC A39622	Arab Cove, Vancouver Is., B.C.	T
UBC A40192	Amphitrite Point, B.C.	T
UBC A40743	Haines Is., Barkley Sound, B.C.	T
UBC A40793	Helby Is., Barkley Sound, B.C.	T
UBC A41214	Meade Islets, Barkley Sound, B.C.	T
UBC A41384	Helby Is., Barkley Sound, B.C.	T
UBC A41415	Fleming Is., Barkley Sound, B.C.	M
UBC A41491	Grappler Inlet, Bamfield, B.C.	T
UBC A41586	Wizard Islet., Barkley Sound, B.C>	F
UBC A41606	Effingham Is., Barkley Sound, B.C.	T
UBC A41607	Effingham Is., Barkley Sound, B.C.	T
UBC A41696	Roquefeuil Bay, Barkley Sound, B.C.	T
UBC A41842	Fleming Is., Barkley Sound, B.C.	F,T
UBC A41843	Fleming Is., Barkley Sound, B.C.	
UBC A41844	Fleming Is., Barkley Sound, B.C.	T
UBC A41946	Clarke Is., Barkley Sound, B.C.	F,T
UBC A42162	Reeks Is., Barkley Sound, B.C.	T
UBC A42436	Fleming Is., Barkley Sound, B.C.	T
UBC A42437	Fleming Is., Barkley Sound, B.C.	T
UBC A42438	Fleming Is., Barkley Sound, B.C.	T
UBC A42439	Fleming Is., Barkley Sound, B.C.	T
UBC A42733	Arab Cove, Vancouver Is., B.C.	
UBC A43525	Diana Is., Barkley Sound, B.C.	T
UBC A43526	Bamfield, B.C.	T

UBC A45812	Bamfield, B.C.	T
UBC A46026	Bamfield, B.C.	F,T
UBC A46960	Sear Is., B.C.	
UBC A53291	Kyuquot Channel, B.C.	T
UBC A53297	Kyuquot Bay, Kyuquot Sound, B.C.	T
UBC A53396	Saanedra Is., Nootka Sound, B.C.	
UBC A53447	Henderson Point, Saanich Inlet, Vancouver Is., B.C.	
UBC A53657	Friendly Cove, Nootka Sound, B.C.	M
UBC A54172	Hotham Inlet, Harmony Is., Jervis Inlet, B.C.	
UBC A55706	Lasqueti Is., B.C.	
UBC A57371	Helby Is., Barkley Sound, B.C.	T
UBC A57429	Moorsam Bluff, Jervis Inlet, B.C.	
UBC A57442	east of Brittain Rock, Jervis Inlet, B.C.	
UBC A57598	mouth of Glacial Creek, Jervis Inlet, B.C.	
UBC A58562	Station Is., B.C.	
UBC A58672	island W of Fox Is., B.C.	
UBC A60374	Diana Is., Barkely Sound, B.C.	T
UBC A60485	Brady's Beach, Bamfield, B.C.	T
UBC A64402	head of Pennell Sound, Queen Charlotte Is., B.C.	T
UBC A64426	head of Pennell Sound, Queen Charlotte Is., B.C.	
UBC A66660	Tasu Sound, Queen Charlotte Is., B.C.	

UBC A66682	Horn Rocks, Tasu Sound, Queen Charlotte Is., B.C.	
UBC A66778	Departure Bay, Vancouver Is., B.C.	
UBC A67220	Cunningham Is., B.C.	
UBC A67249	Bachelor Bay, B.C.	
UBC A67446	Kirby Point, Diana Is., Barkley Sound, B.C.	F, T
UBC A69432	Cape Suspiro, Alaska	T
UBC A69433	Cape Suspiro, Alaska	
UBC A69434	Kanaka Bay, San Juan Is., Washington	T
UC 93572	Moss Beach, San Mateo Co., California	F HOLO-
		TYPE
UC 276633	Moss Beach, San Mateo Co., California	F
UC 296689	Moss Beach, San Mateo Co., California	F, T
UC 305332	Moss Beach, San Mateo Co., California	F
UC 305364	Pebble Beach, Monterey, California	F
UC 305371	Pescadero, California	F
UC 305373	Pescadero, California	M
UC 1452112	Moss Beach, California	F
private		
collection of		
J.R. Waaland:		
1583	Kanaka Point, San Juan Is., Washington	T

Gelidium robustum

UBC A1448	Moss Beach, California	T
UBC A7861	Shoal B, Victoria	T
UBC A37790	Asilomar Point, California	
UBC A47163	Palos Verdes, California	
UBC A47164	Santa Catalina Is., California	
UBC A50638	Point Pinos, Monterey Peninsula, California	
UBC A58591	Santa Cruz Is., California	T
UBC A60676	Pedros Blancas, California	
UBC A62199	Carpinteria Reef, California	F
UBC A63553	Monterey, California	
UC 294572	near Ensenada, California	T HOLO- TYPE
UC 395419	San Pedro, California	F
UC 647822	White's Point, San Pedro, California	F
UC 756464	Punta Santa Rosalia, Baja California, Mexico	
UC 756469	Punta Santa Rosalia, Baja California, Mexico	
UC 756470	Rosario, Baja California, Mexico	
UC 940173	Natividad Is., Baja California, Mexico	F
UC 1451987	Portuguese Bend, San Pedro, California	F

Gelidium sinicola

UC 276620	Point Cavallo, Marin Co., California	HOLOTYPE
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Gelidium vagum

TNS 25817	Harutachi, Hidaka, Japan	T
TNS 25823	Hideshima, Iwate-ken, Japan	T
TNS 25824	Ta-no-hama, Iwate-ken, Japan	F
TNS 25825	Ta-no-hama, Iwate-ken, Japan	F
TNS 25847	Same, Aomori-ken, Japan	T
UBC A56807	Muroran, Hokkaido, Japan	T
UBC A64965	Ladysmith, Vancouver Is., B.C.	T

Pterocladia caloglossoides

UBC A12295	McLean Is., Vancouver Is., B.C.	
UBC A19667	Bunsby Is., Vancouver Is., B.C.	
UBC A28937	Orlebar Point, Gabriola Is., B.C.	T
UBC A29576	Orlebar Point, Gabriola Is., B.C.	T
UBC A30401	Whiffen Spit, Sooke, Vancouver Is., B.C.	
UBC A31251	Whiffen Spit, Sooke, Vancouver Is., B.C.	
UBC A33545	Arab Bay, Vancouver Is., B.C.	
UBC A37605	Inner Bajo Reef, Nootka Is., Vancouver Is., B.C.	
UBC A39126	Lawn Point, Vancouver Is., B.C.	
UBC A39127	Lawn Point, Vancouver Is., B.C.	
UBC A61245	Diana Is., Barkley Sound, B.C.	T

UBC A64648	head of Rennell Sound, Queen Charlotte Is., B.C.
UBC A69423	Cape Suspiro, Alaska
UBC A69424	Cape Suspiro, Alaska
UBC A69425	Cape Suspiro, Alaska
UBC A69426	San Clemente Is., Alaska
UBC A69427	Sea Otter Sound, Alaska
UBC A69428	Sea Otter Sound, Alaska
UBC A69429	Sea Otter Sound, Alaska
UBC A69430	Sea Otter Sound, Alaska
UBC A69431	SeaOtter Sound, Alaska
WTU 248018	Mitchell Bay, San Jaun Is., Washington

APPENDIX 2. PROCEDURE FOR EMBEDDING MATERIAL IN JB4 METHACRYLATE

Fixation

2.5% glutaraldehyde in Sorensen's phosphate buffer (pH 7.2) (1:1)

4 h, room temperature

↓

3 10 min washes in Sorensen's buffer

↓

Dehydration

10%, 25%, 40%, 50%, 70%, 80%, 90%, 100%, 100%, 100% MeOH

each step 10 min

10% - 80% in refrigerator, 90%, 100% at room temperature

↓

Infiltration

1 part catalyzed solution A : 3 parts 100% MeOH

1 h

↓

1 part catalyzed solution A : 1 part 100% MeOH

4 h

↓

3 parts catalyzed solution A : 1 part 100% MeOH

8 h or overnight, refrigerator

↓

100% catalyzed solution A

overnight, refrigerator



Embedding

1 part solution B (activator) : 25 parts catalyzed solution A

poured into mold ,

tissue arranged in mold,

capped with a stub,

hardened in refrigerator, overnight

APPENDIX 3. PREPARATION OF TETRASPORANGIAL AND SPERMATANGIAL MATERIAL FOR TRANSMISSION ELECTRON MICROSCOPY

Fixation

2.5% glutaraldehyde in Sorensen's phosphate buffer (pH 7.2) (1:1)

tetrasporangial: 4 h, room temperature/**spermatangial:** 7 h, refrigerator

↓

3 10 min washes in Sorensen's buffer

↓

Osmication

1% OsO₄ in buffer (1:1)

tetrasporangial: 18-19 h, refrigerator/**spermatangial:** 16 h, refrigerator

↓

3 10 min washes in buffer

↓

Dehydration

10%, 25%, 40%, 50%, 70%, 80%, 90%, 100%, 100%, 100% MeOH

each step 10 min

10% - 80% in refrigerator, 90%, 100% at room temperature

↓

Infiltration

25%, 50%, 75%, 100% propylene oxide in MeOH

20 min, room temperature

↓

Embedding

10% Spurr's epoxy resin in propylene oxide

overnight, refrigerator, on rotator

↓

25%, 40%, 55%, 70%, 80%, 90%, 100%, 100%, 100% Spurr's in propylene oxide

each step $\frac{1}{2}$ day at room temperature on rotator, or overnight in refrigerator

↓

Polymerization

in Spurr's epoxy resin

under 18 psi vacuum $\frac{1}{2}$ day, then

600 min, 70°C, 18 psi