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An investigation of cystocarp development in *Gelidium pteridifolium* with a revised description of the Gelidiales (Rhodophyta)

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The Gelidiales possess a unique combination of characters that set them apart from all other orders of red algae. Our observations have revealed features in the development of the female reproductive system that differ from reports of previous workers. We find that in *Gelidium pteridifolium* nutritive filaments are initiated prior to carpogonial elongation; functional carpogonia are intercalary; a fertilized carpogonium becomes multinucleate as it cuts off gonimoblast initials; nuclei in the cortical filaments borne on the carpogonium divide and enlarge following fertilization and pit plugs break down forming channels between the carpogonium and adjoining cortical filaments; terminal gonimoblast cells first fuse with the terminal cells of nutritive filaments and later unite with the fusion cells themselves; unfused, uninucleate gonimoblast cells cut off the first carposporangia, and multinucleate fusion cells produce a second series of carposporangia later on. The auxiliary cell concept is reviewed as it has been applied to Gelidiales. We have provided a revised description of the order Gelidiales and discuss its status and possible taxonomic affinities.

INTRODUCTION

The morphology of the female reproductive system in the Gelidiaceae has been investigated previously by Kylin (1928), Dixon (1959), and Fan (1961). In the present study we reconstruct the sequence of events leading to the formation of the carpogonium and nutritive filaments before fertilization and the development of the carposporophyte and cystocarp after fertilization in *Gelidium pteridifolium* Norris, Hommersand and Fredericq (1987). Our observations are compared with those of previous workers in the light of longstanding controversies regarding fusions of the fertilized carpogonium, presence or absence of auxiliary cells, and fusions of gonimoblast with nutritive filaments in the Gelidiaceae. Finally, we provide a revised description of the order Gelidiales and comment on its status and possible taxonomic affinities.

MATERIALS AND METHODS

The principal material used in this study were female plants of *Gelidium pteridifolium* Norris, Hommersand and Fredericq (1987) collected in the drift at Sharks Cove, The Kowie, Port Alfred, South Africa, by Max H. Hommersand, 14 December 1977, and preserved in 5% formalin/seawater. Additional observations were made on female material of other selected species of *Gelidium*, *Pterocladia*, and *Suhria* for purposes of comparison.

Periclinal, longitudinal, and transverse sections of fertile pinnules were made by hand with a platinum-chrome double-edged razor blade. Sections were stained on the slide either with aniline blue (Papenfuss 1937), a general stain for proteins, or aceto-iron-haematoxylin-chloral hydrate (Wittmann 1965; Coomans 1986), which stains the nuclei, cytoplasm and pit connections in formalin-preserved material. Slides stained with aniline blue were permanently mounted in 50% Karo (registered brand) corn syrup. Sections to be stained with Wittmann's stain were air dried and re-expanded briefly in distilled water to remove salt crystals, after which the water was removed with tissue paper. Two drops of the stain were pipetted onto the sections and a coverslip applied. Sections were stained for 30 min to 3 h during which time the staining process was fol-

lowed under a microscope. Slides were destained with 45% acetic acid solution which was flushed under the coverslip at one end and drained off with tissue paper at the other. When appropriate staining was achieved one drop of Hoyer's mounting medium (Stevens 1974) diluted 1:1 with distilled water was added along one edge of the coverslip and left for a day. Any excess Hoyer's medium was wiped from the edges of the coverslip and the coverslip ringed with transparent nail polish. Bright field and Nomarsky interference phase contrast pictures were taken with a Zeiss Photomicroscope III using Pan X film. Pictures were taken during the staining process or after transfer to the permanent mounting medium.

HISTORICAL BACKGROUND

Kützing (1843, pp. 390, 405) established the family Gelidiaceae (as 'Gelidieae') to include pinnately branched algae that are tough and cartilaginous with a fibrous interior. Reproductive characters were not emphasized. J. Agardh (1851, p. ix, 1852, p. 464, 1876, p. 543) emended the description of the Gelidieae to include only algae having the following cystocarpic features: (i) cystocarp within a raised, hemispherical pericarp with either 1 locule or 2 opposite locules separated by a longitudinal septum and bearing placentae along the base of the locule or paired along the sides of the septum, (ii) carposporangia-bearing filaments united or free, consisting of many short fascicles (includes present-day nutritive filaments) arranged in dense rows along the wall from the basal placenta interspersed with columns of sterile filaments extending to the pericarp, (iii) fertile branchlets bearing clavate to obovate carposporangia one to few per segment in terminal segments of the branchlets. Structural features recognized by J. Agardh are still accepted, although interpretations may have changed. Bornet and Thuret (1876, pl. 20) illustrated both the *Pterocladia*-type (1 locule) and the *Gelidium*-type (2 locules) cystocarp.

The concept and circumscription of the Gelidiaceae was greatly modified by Schmitz & Hauptfleisch (1896, pp. 305, 341) when they expanded the family to include Florideae (= Florideophycidae) in which one or more gonimoblasts develop directly from the fertilized carpogonium, usually after fusion with nearby cells, and develop into filaments that ramify among the inner cortical cells in the vicinity of the central axis and frequently unite with individual sterile thallus cells before branching outwardly to form a layer of filament tips (referred to as a 'hymenium') that bear carpospores terminally. The revised circumscription included genera that are now spread among five orders of red algae. It was not until Feldmann & Hamel (1934) removed both *Caulacanthus* and *Wurdemannia* from the Gelidiaceae that the original concept of J. Agardh was fully restored.

Historically, the Gelidiaceae have played a pivotal role in the controversy over what does, and what does not, constitute an auxiliary cell in red algae. Indeed, the term 'Auxiliarzellen' was first used by Schmitz in connection with his discussion of carposporophyte development in the 'Gelidieen' group. Schmitz (1883, p. 228) commented that in *Pterocladia* the branches of the 'ooblast' filaments meander through the cell masses of the small-celled tissue and attach many times to individual cells of this tissue which are highly rich in cell contents. Speaking of the 'Gelidieen' group as a whole, Schmitz (1883, p. 229) called these cells auxiliary cells, which often have as their only function support of the vigorous and luxurient development of the ooblast filaments.

At the same time that he erected the order Gelidiales on somewhat nebulous grounds, Kylin (1923, p. 132) argued that in Gelidiaceae the 'auxiliary cell' of Schmitz is only a 'nurse cell' since the point of gonimoblast initiation is the carpogonium, the same as in 'Nemalionaceae' (= Nemaliales *sensu* Kylin 1923). Subsequently, Kylin (1928) provided the first detailed description of the development of the carpogonium and 'nurse cells' (= nutritive filaments) in a species of *Gelidium*. He was unable to ascertain whether or not the gonimoblast filaments connect with the nurse cells by pit connections, fuse with them directly, or alternatively can extract nutrients through the cell walls of the content-rich nurse cells. Kylin (1928, p. 29) again concluded that there is no auxiliary cell in Gelidiaceae comparable to that present in higher Florideophycidae which serves as the starting point of gonimoblast formation. On the other hand, he considered the differences in fruit development between Gelidiaceae and 'Nemalionaceae' to be so great as to warrant their realignment in separate orders. Additionally, he commented that the 'Nemalionaceae' are haplobiontic whereas the Gelidiaceae are diplobiontic. Subsequently, Kylin

(1932) used this feature as his key distinguishing character for separating the Nemaliales from the Gelidiales.

Dixon (1959) observed that the carpogonium is cut off laterally and is sessile, 'although in some examples the wall does not develop and the carpogonium is intercalary'. He concluded that the 'gonimoblast does not develop directly from the unchanged carpogonium, as was thought by Kylin, but a swollen multinucleate cell of irregular outline is formed first, either from the carpogonium alone, or by the fusion of the carpogonium and certain neighboring cells'. Since these fusions did not appear to be obligatory and since the diploid nucleus is not transferred to any of the vegetative cells in question, Dixon argued that auxiliary cells are absent in Gelidiaceae.

Fan (1961) investigated carposporophyte development in species representing five genera in the Gelidiaceae. He established that the features of the female reproductive system are conservative and characteristic of the family as a whole. Fan showed that nutritive filaments usually develop from the basal cells of every cell row of the third order in the fertile area and that the carpogonium initial is usually the second basal cell of a cell row of the third order. He reported that after fertilization the carpogonium fuses with the supporting cell by a widening of the pit connection between them and that gonimoblast cells fuse with cells of the nutritive filaments, probably directly, without forming connecting cells. Fan (1961, p. 344) interpreted cells of the nutritive filaments in the Gelidiaceae as 'nutritive auxiliary cells' *sensu* Papenfuss (1951).

Dixon (1961) argued that the original basis on which Kylin separated the Gelidiaceae from the Nemaliales, namely the presence of a diplobiontic life history in the former and a haplobiontic life history in the latter, is no longer valid in view of the numerous reports of irregularities in the life histories in Gelidiaceae and the discovery of both sexual and tetrasporic plants in the life histories of many genera retained in the Nemaliales. Dixon's circumscription of the Nemaliales, which included the Gelidiaceae, has been frequently adopted in floras and texts during the past two decades.

Fan (1961) provided a comprehensive historical review of the classification of the Gelidiaceae and the Gelidiales, and Santelices (1974) has given a full résumé of the taxonomy, morphology, ecology and economic importance of the Gelidiales.

RESULTS

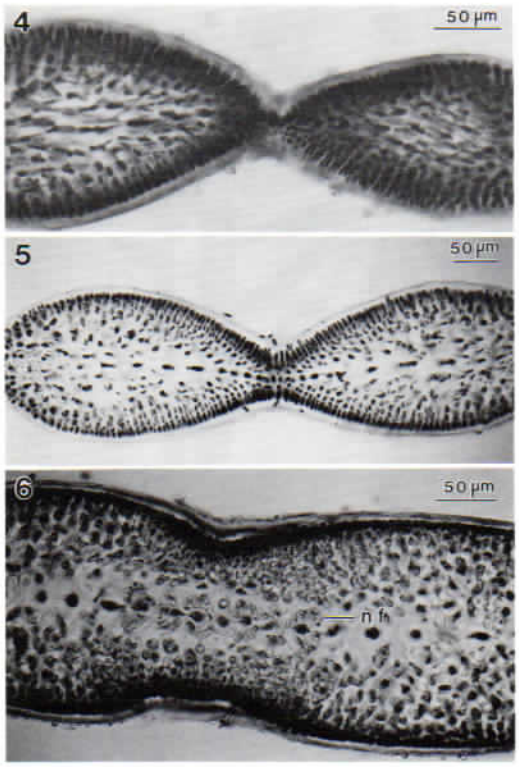
Mature female plants of *Gelidium pteridifolium* from Port Alfred, South Africa, stand 30–50 cm high with one to several primary axes arising from a branched, stoloniferous holdfast. Each axis bears up to four orders of pinnately branched indeterminate vegetative laterals that are largely denuded below and beset with alternate to subopposite, distichously arranged rows of determinate fertile branchlets above (Fig. 1). The general outline of major axes is pyramidal; however, branches of any order may be deciduous leading to irregularities in branching pattern. Fertile female branchlets are once or twice compound and beset with pinnately to irregularly arranged pinnules. Development is acropetal with the youngest, unfertilized pinnules at the tips and older, postfertilization stages becoming evident below as the cystocarps expand, forming raised circular to oval areas along the midline on both sides of the pinnule (Fig. 2). A young female pinnule is easily distinguished from a vegetative tip because the apex is notched and a longitudinal furrow extends down the middle of the pinnule on both sides for about two-thirds of its length (Fig. 3). At maturity, a fertile pinnule may form one, two, or occasionally three cystocarps along the midline and is usually terminated by a sterile apiculate tip, or the tip may regenerate a new, compound branchlet (Fig. 2). Cystocarps are biconvex with a circular plug or papilla at the center of the pericarps on either side (Fig. 3). At maturity the central plug in each pericarp breaks down forming a pair of ostioles facilitating carpospore release. Later, the cystocarps dehisce leaving circular to oval spaces in the pinnule (Fig. 2). Finally, the pinnules and fertile branchlets abscise.

Growth takes place by means of a transversely dividing apical cell. In vegetative apices, which are ordinarily acute, the apical cell is prominent, but in female pinnules it comes to lie in a notch overtopped on both sides by the more rapidly growing thallus margin. Growth of the cortex directly above and on either side of the central axis of a female pinnule is likewise retarded so that furrows form in the median anticlinal plane on each side of the pinnule behind the apical notch. The reproductive structures develop within and alongside these furrows. Anatomically, the area adjacent to the fertile central furrow contrasts sharply with the surrounding vegetative tissue, as is seen in a series of transverse sections taken close to the apex (Figs 4–6). Tissue

Figs 1–3. *Gelidium pteridifolium.*
Fig. 1. Habit of female plant.
Fig. 2. Branchlet with young and mature female pinnules and pinnules in which cystocarps have dehisced (arrowhead). Note regenerating tips (arrows).
Fig. 3. Young pinnules with apical notch and median, anticlinal furrow, older pinnule with developing cystocarp and papilla (arrow).

on either side of the apical initial contains rhizoids and resembles vegetative cortex and medulla (Fig. 4). Five to seven segments behind the apex, the fertile area consists of rows of cells belonging to filaments of the second order that are initially spaced close together on both sides of the central axis, and are readily distinguishable from vegetative tissues toward the margins (Figs 5, 8). Rhizoidal filaments are present in the medulla of the vegetative tissue along the margins and are absent in the fertile central region. Nutritive filaments are initiated from basal cells of filaments of the third order at about the level of the seventh axial segment behind the apex. By the tenth segment the nutritive filaments envelop nearby cells of the second order (Fig. 6). At maturity, the fertile area extends laterally across seven or eight vertical files of cells belonging to filaments of the second order on either side of the central axis. Axial cells themselves do not bear nutritive filaments.

Stages in the development of vegetative and reproductive tissues at the apex of the female pinnule can be seen in oblique and median periclinal sections (Figs 7, 8). The boundary between the vegetative margin and the fertile central area is clearly delimited. As seen in a median periclinal section (Fig. 8), second-order filaments extend laterally at an angle of about 30° on both sides of the central axis. Each cell is connected in vertical files by secondary pit connections. Secondary pit connections form about four or five segments behind the apex and before the first nutritive filament initials are cut off as illustrated by Fan (1961, fig. 3a). Every cell of a second-order filament in the fertile area has nutritive



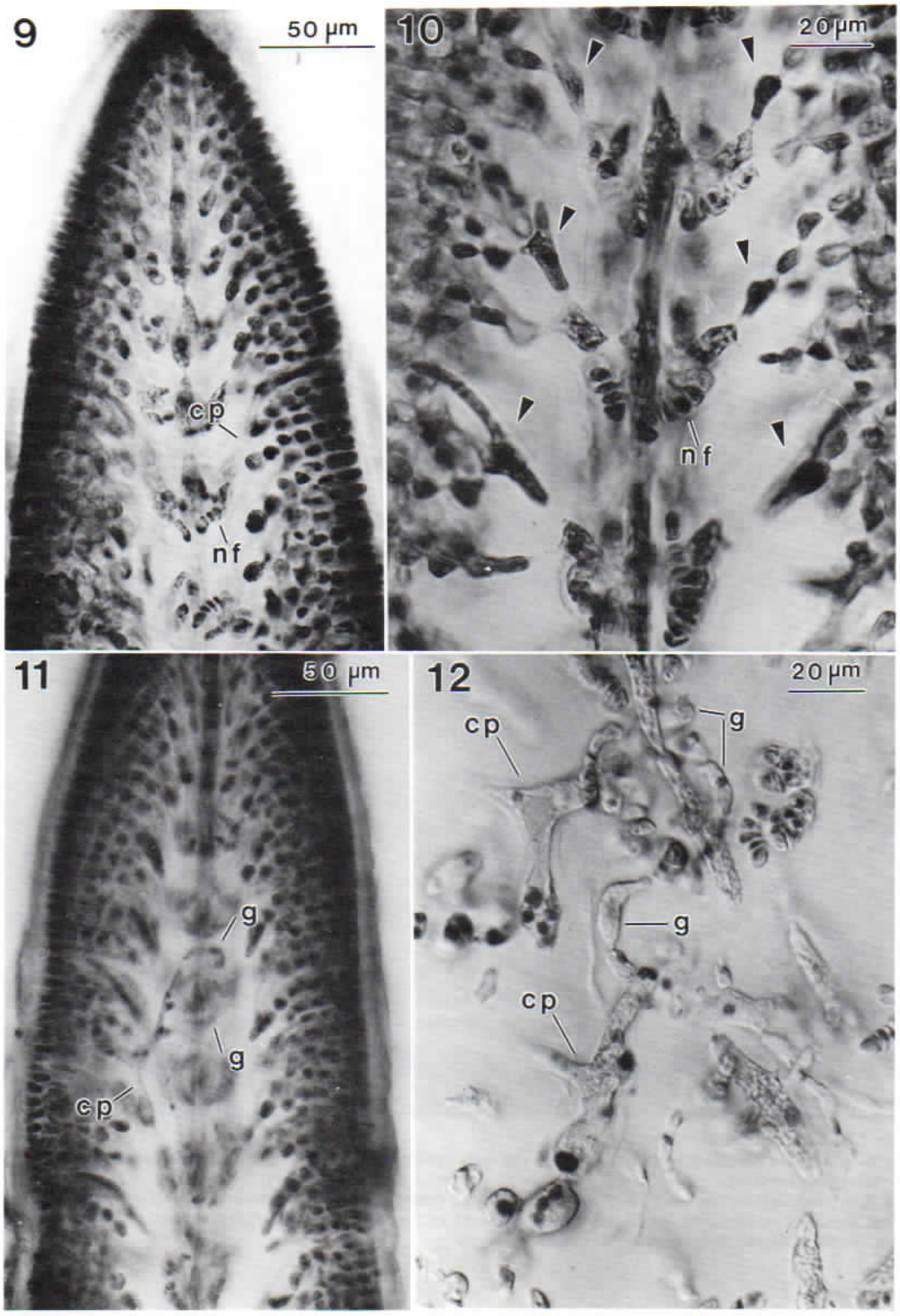
Figs 4–6. *Gelidium pteridifolium.*
Fig. 4. Cross-section of young female pinnule at level of apical cell.
Fig. 5. Cross-section of young female pinnule 6–7 segments behind apex.
Fig. 6. Cross-section of female pinnule about 10 segments behind apex with nutritive filaments (nf).

filaments associated with it and clusters of nutritive filaments lie in a gridwork of horizontal rows and longitudinal files. Nutritive filaments develop from the lower and lateral faces of basal cells of filaments of the third order. Normally from one to three initials are cut off, each of which can produce a descending filament up to ten cells long.

Carpogonia begin differentiating along the furrows on either side of the central axis at the time the nutritive filaments are initiated; however, the trichogynes elongate only after the nutritive filaments are well developed (Fig. 7). In our material the carpogonial initial was always an intercalary suprabasal cell of an anterior filament belonging to the third order (Figs 9, 10). It becomes densely filled with cytoplasm and the trichogyne curves obliquely toward the apex reaching the thallus surface at about the same time that the nutritive filaments have reached their full length. The carpogonium remains an intercalary cell at maturity. We have not seen any instance in which the lateral protuberance of the carpogonium has been cut off as a separate cell in *Gelidium pteridifolium*.

Many carpogonia can be fertilized in a single fertile area of a pinnule, and while some develop normal gonimoblasts, others abort. A cystocarp is thus almost always a composite structure containing several individual carposporophytes. For example, while only one carpogonium and developing gonimoblast is seen in Fig. 11, two more were present in this section out of focus. The squash preparation in Fig. 12 shows two carpogonia with normally developing gonimoblasts side by side. Fertilized carpogonia that have aborted are easily distinguishable from normally developing ones. An aborted carpogonium becomes greatly and irregularly swollen (Fig. 14). Few gonimoblast initials are cut off and these tend to remain one-celled. Such a carpogonium becomes multinucleate with many small nuclei, all of about the same diameter. In normal development, on the other hand, the carpogonium tends to retain its shape following fertilization, enlarging slightly and producing a mixture of relatively few large and small nuclei. It is narrowly triangular due to retention of part of the trichogyne (Figs 11, 12) and produces long, branched distal processes (Figs 13, 15, 17, 18). Uninucleate gonimoblast initials are budded off distally, usually anterior to the site of the emerging trichogyne (Figs 11, 12, 15) or immediately below it (Fig. 13). The intercalary part of the carpogonium, the foot, did not produce gonimoblast filaments. A nucleus at the tip of a process divides, with one daughter nucleus passing into the gonimoblast initial and the other remaining in the carpogonial process, usually near the pit connection. As the carpogonium enlarges and cuts off gonimoblast initials, the number of nuclei tend to increase. From this observation we conclude that not all nuclear divisions are associated with gonimoblast initiation. Nuclear volume, which is variable, is possibly related to the physiological state of the nucleus. Sometimes the first cell cut off from the carpogonium distends, becomes multinucleate, and serves as a secondary site for the production of uninucleate gonimoblast initials.

At the time of gonimoblast formation, the two cortical files distal to the carpogonium become highly modified. Cortical cells close to the carpogonium become binucleate or occasionally


9
50 µm
cp
nf
10
20 µm
nf
11
50 µm
g
g
cp
12
20 µm
cp
g
g
cp

multinucleate, and the nuclei enlarge up to three to four times their original diameter (Figs 12–16). More distal cortical cells remain uninucleate and unmodified. Fusions commence between the cortical cells and the foot of the carpogonium. In every instance the pit plug breaks down and fusion proceeds through the center of the pit connection rather than around it. As cytoplasm becomes continuous through the pit connection a ring of septal material remains intact at the periphery (Fig. 16). Even at maturity the outline of the fused cortical cells is distinct, bordered by thickened, highly stainable wall material (Fig. 17). The expanded carpogonium, the remains of the trichogyne, and the fused cortical filaments are all clearly visible even after the gonimoblast is well along in its development.

Early growth of the gonimoblast filaments is directed towards the nutritive filaments (Figs 11–13, 15). Branching of the gonimoblast generates a network as gonimoblast filaments ramify along the rows and files of nutritive filaments (Figs 8, 17). Fusions between gonimoblast and nutritive cells do not begin immediately, and an extensively branched system of gonimoblast filaments may develop before fusions take place. At some point gonimoblast filaments cut off cells laterally that elongate and approach the terminal cells of the nutritive filaments. The appearance of an elongated gonimoblast cell is especially evident in material stained with aniline blue (Figs 18, 19). In such preparations the short-celled, densely staining filaments are nutritive filaments and the thin, long cells that link up with them are gonimoblast cells. Cells of nutritive filaments are usually uninucleate at this stage. The nuclei stain deeply with either Wittmann's stain or aniline blue and occupy a large part of each cell (Figs 17, 18).

Followed step by step (Figs 20–25), gonimoblast filaments are seen to bud off small initials laterally which grow directly toward the terminal cell of a nutritive filament (Figs 20, 21). The terminal portion of the gonimoblast filament clasps the terminal nutritive cell and expands around it before wall breakdown and fusion take place (Figs 22, 23). Following fusion, the gonimoblast cell is commonly binucleate, rarely trinucleate, suggesting that the presumably diploid gonimoblast nucleus divides immediately after fusion and the haploid nucleus of the nutritive cell degenerates (compare Figs 24 and 26). Cytokinesis commonly appears to be initiated immediately after nuclear division but a pit plug is not formed (Fig. 26). A terminal gonimoblast cell may fuse with any terminal nutritive cell, including one-celled laterals of nutritive filaments. Often a gonimoblast cell will fuse with two nearby terminal nutritive cells creating a branched fusion cell (Fig. 25). In no instance have we seen an initial fusion occurring between gonimoblast filaments and intercalary nutritive cells in *Gelidium pteridifolium*. Usually the two or three nutritive cells connected to the fused terminal cell become binucleate (Figs 24, 26). Pit connections between cells of the nutritive filaments remain small and appear to be unmodified at this stage.

Once the terminal gonimoblast and nutritive cells have fused, the remaining unfused gonimoblast cells cut off the first carposporangial initials (Figs 19, 26, 27). At this stage the reproductive apparatus consists of a reticulum of branched nutritive filaments, small fusion cells, and gonimoblast cells bearing young carposporangia (Figs 27, 30). Additional fusions between gonimoblast cells, nutritive cells and existing fusion cells further expand the network and lead to the formation of multinucleate centers (Figs 28, 29). The later-formed multinucleate cells cut off additional carposporangial initials. A transverse section of a young cystocarp at this stage contains a mixture of carposporangia of varying sizes, usually in two distinct size categories (Figs 31, 32). The larger carposporangia, which were cut off before the secondary fusions occurred, are attached to uninucleate gonimoblast cells. The smaller, younger carposporangia arise from multinucleate cells that are the products of secondary gonimoblast fusions (Fig. 33). As both crops of carposporangia develop, the nutritive cells lose

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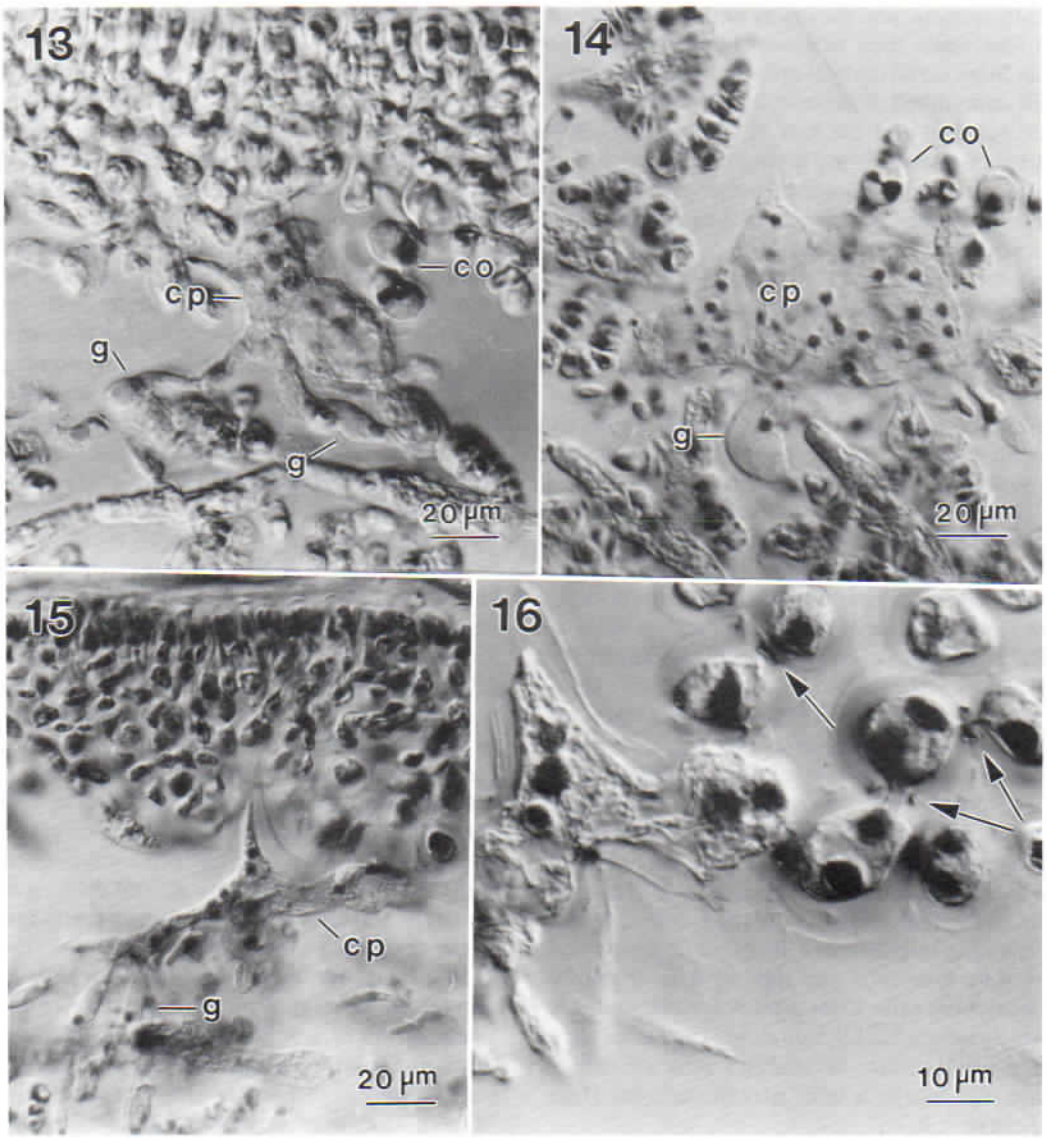
Figs 9–12. *Gelidium pteridifolium.*

Fig. 9. Longitudinal section of fertile tip with developing nutritive filaments (nf) and carpogonia (cp) with trichogynes.

Fig. 10. Intercalary carpogonia (arrowheads) on suprabasal cells of third-order filaments initiating trichogynes. Nutritive filaments (nf) are well developed.

Fig. 11. Fertilized carpogonium (cp) with processes bearing gonimoblast (g) in vicinity of nutritive filaments.

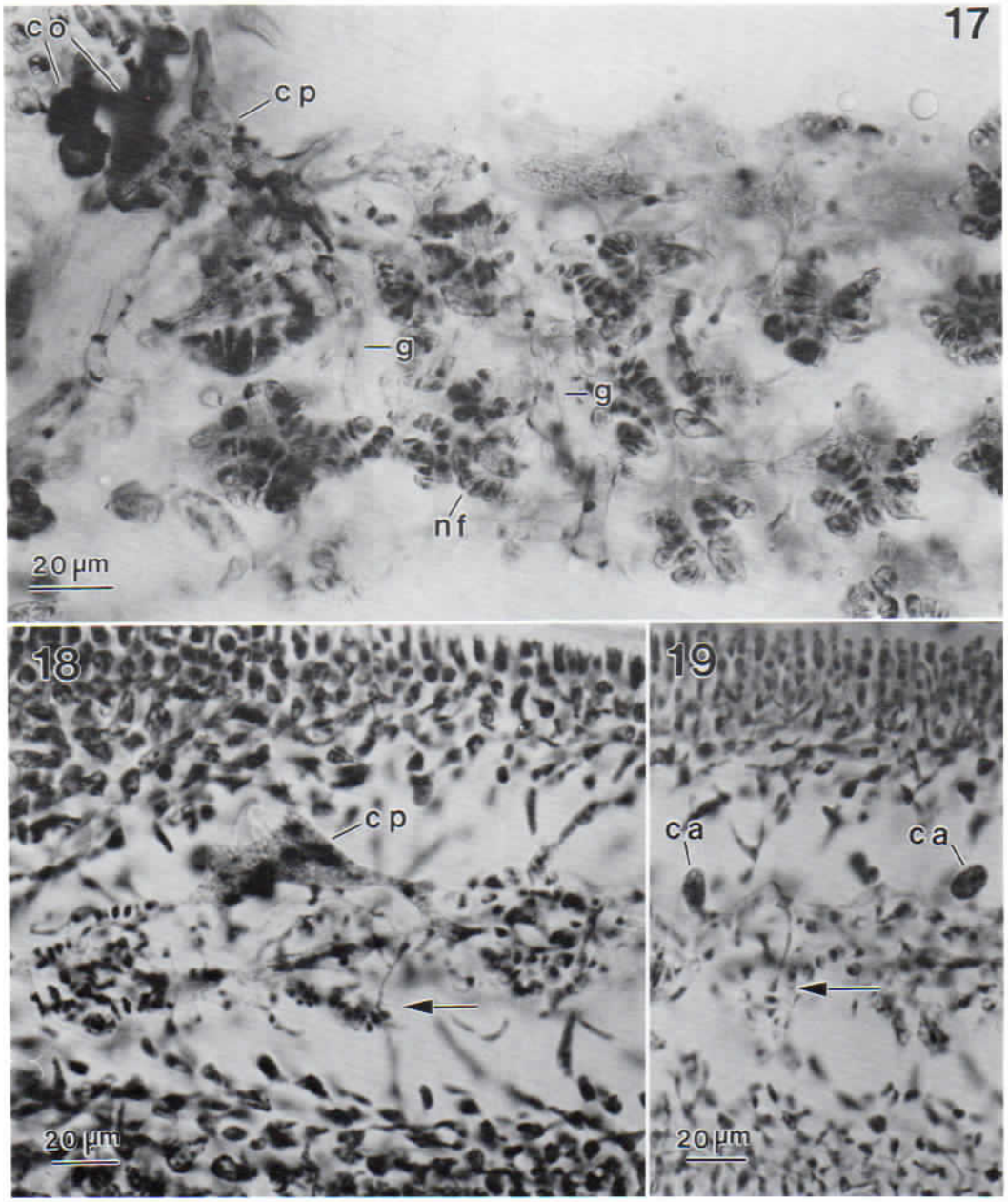
Fig. 12. Two adjacent carpogonia (cp) with processes bearing gonimoblast filaments (g). (File of second-order filaments partly disrupted.)



Figs 13–16. *Gelidium pteridifolium.*
Fig. 13. Attached multinucleate carpogonium (cp) bearing modified cortical filaments (co) and two young gonimoblast filaments (g).
Fig. 14. Distended multinucleate carpogonium (cp) that has probably aborted, with cortical filaments (co) and a few one-celled gonimoblast initials (g).
Fig. 15. Detached multinucleate carpogonium (cp) bearing several inwardly growing gonimoblast filaments (g). (Gonimoblast cells are uninucleate.)
Fig. 16. Enlarged view of multinucleate carpogonium bearing pair of cortical filaments in which innermost cells are binucleate. Fusion has occurred through septal plugs of cortical cells (arrows).

their staining properties and become vacuolate (Fig. 32). Pit connections between adjoining nutritive cells appear to break down. An enlarged view of a transverse section shows a row of cells of second-order filaments in the center surrounded by a network of small nutritive cells, larger gonimoblast cells, fusion cells and elongating carposporangia (Figs 32–34).

The cavity inside the cystocarp enlarges gradually beginning with the earliest stages of gonimoblast formation. It is probable that mucilaginous material is secreted into this space,

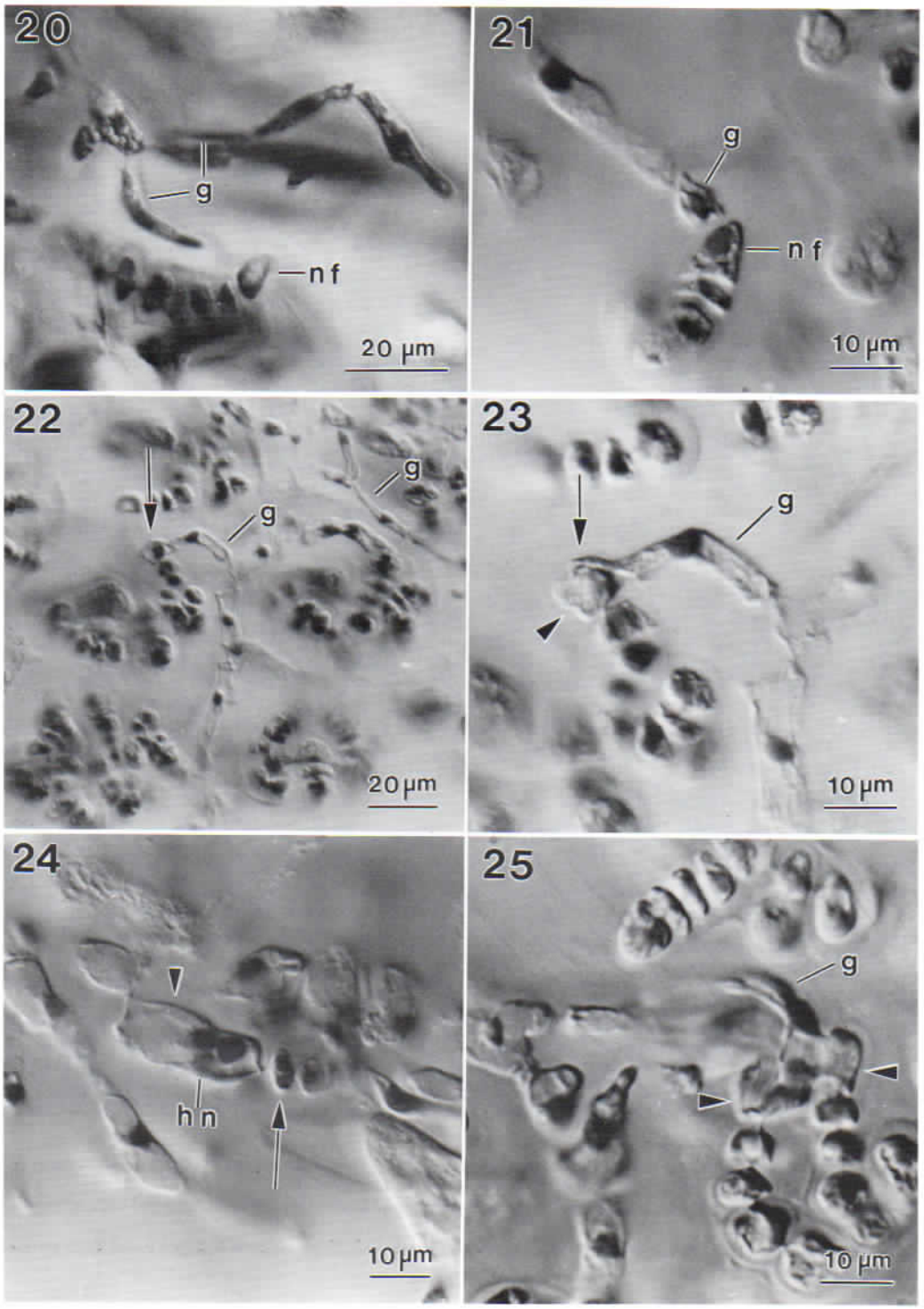


Figs 17–19. *Gelidium pteridifolium.*

Fig. 17. Fertile area in which cortex has been stripped away to reveal carpogonium (cp), fused cortical cells (co), and gonimoblast (g) ramifying between rows of nutritive filaments (nf).

Fig. 18. Carpogonium (cp) and gonimoblast filaments at stage of fusion (arrow) with terminal cells of nutritive filaments.

Fig. 19. Gonimoblast filaments bearing young carposporangia (ca). Gonimoblast cell has fused with terminal cell of nutritive filament (arrow).



Figs 20–25. *Gelidium pteridifolium.*

Fig. 20. Stage showing branching and directed growth of gonimoblast filament (g) in vicinity of nutritive filament (nf).

Fig. 21. Terminal gonimoblast cell (g) prior to contact with terminal cell of nutritive filament (nf).

expanding the cystocarp. Inner cortical cells elongate during the expansion process (Figs 18, 30, 31, 34). At maturity the cystocarp is biconvex with a central partition and paired placentae bearing carposporangia. Potential ostiolar regions develop on opposite sides (Fig. 34). Initially these are plugged and sometimes papillate (Fig. 3).

DISCUSSION

Carpogonium and carposporophyte development

Our findings suggest that the formation of the carpogonium and nutritive filaments before fertilization and the development of the carposporophyte and cystocarp after fertilization follow a prescribed sequence of events. In *Gelidium pteridifolium* nutritive filaments develop before the trichogyne elongates and the carpogonium is always intercalary. This is in contrast to the observations of Kylin (1928), Dixon (1959) and Fan (1961), all of whom report that the carpogonium develops first and is commonly cut off as a sessile, one-celled lateral. Because of the differences in observations, we examined six additional species belonging to *Gelidium* (3), *Suhria* (1) and *Pterocladia* (2). In some material it was evident that the intercalary carpogonium had elongated rapidly, at a comparatively early stage of development of the nutritive filaments. We frequently encountered one-celled carpogonia that had been cut off laterally leaving an intercalary supporting cell, especially in older pinnules with developing carposporophytes. Sessile, lateral carpogonia all appeared to be non-functional. We saw no evidence of fusion of a lateral carpogonium with its supporting cell, and no indication that these cells become multinucleate or take part in later postfertilization events. It is true that a normally developing fertilized carpogonium may have a constriction between the expanded portion below the trichogyne and the basal part that remains attached to the cortical cells as illustrated by Fan (1961, figs 4b, 6c), but we saw no evidence of the breakdown of an adjoining pit connection or fusion through it. The neck of the distended, fertilized carpogonium appears, instead, to be simply the unexpanded region next to the intercalary foot or base.

In agreement with Dixon (1959) we find that a fertilized carpogonium develops long, multinucleate, nonseptate processes before cutting off gonimoblast initials. Mostly, these extend anteriorly, toward the apex of the pinnule and produce gonimoblast initials from their tips. The only fusions we have seen at this stage occur between the carpogonium and cells of adjoining cortical filaments. These take place through the pit connections as described in the Results section, and ordinarily remain narrow until after the full complement of gonimoblast initials has been cut off. We have not seen any indication of random fusions involving the carpogonium as reported by Dixon (1959, figs 3b, e), and we interpret his figures as representing stages in the fusion of cortical cells similar to those we have described. Fan (1961) has said that the pit connections between the supporting cell and the vegetative cells borne on it may widen later, but that does not always occur. We suspect that fusions with the cortical cells are a regular feature of postfertilization carpogonium development and that the differences in observation relate to the extent of breakdown of the septal ring constricting the passageway between cells. Although the connecting region between the cortical cells and the carpogonium usually broadens at a late stage, the modified vegetative nuclei, which are readily distinguishable by their size and staining properties, appear to remain inside the cortical cells even when extensive fusions have taken place. We have observed that in *Suhria* cortical filaments adjoining the carpogonium fuse into two large, lobed, multinucleate fusion cells containing enlarged, densely staining nuclei. These are the lobes illustrated by Fan (1961, fig. 7c) that, in reality, are part of the cortical system.

In Gelidiaceae the carpogonium may cut off cells that become multinucleate and highly vacuolate. These serve as secondary centers for the

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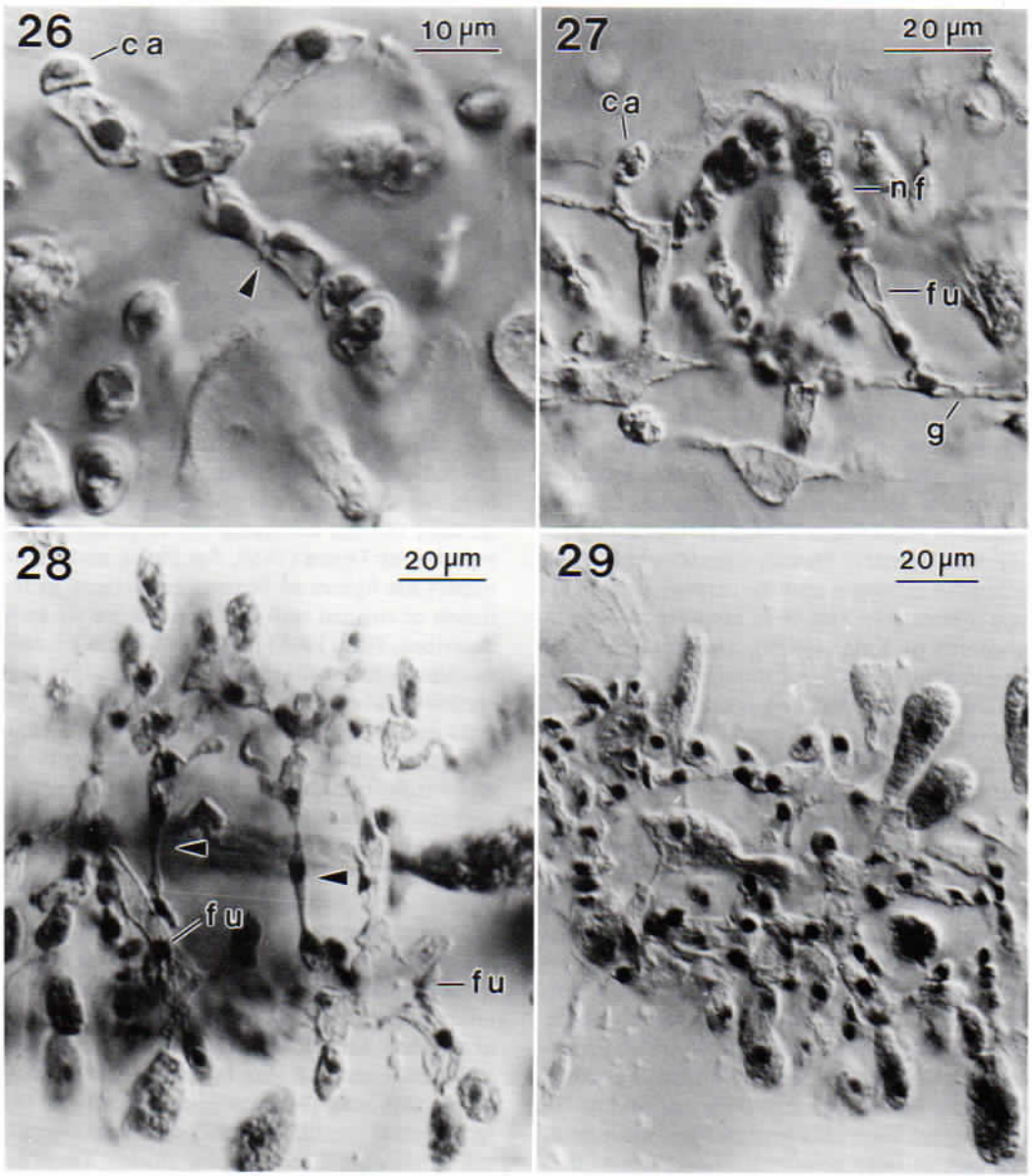
Fig. 22. Branching of gonimoblast filaments (g) and fusion (arrow) with terminal cell (arrowhead) of nutritive filament.
Fig. 23. Enlarged view of area in Fig. 22 showing contact and initial fusion (arrow) of terminal gonimoblast cell (g) with terminal cell of nutritive filament (arrowhead).
Fig. 24. Fused gonimoblast and terminal nutritive cell (arrowhead). Diploid nucleus has divided and haploid nucleus (hn) is degenerating. Subterminal nutritive cell is binucleate (arrow).
Fig. 25. Gonimoblast cell (g) fused with two adjacent terminal nutritive cells (arrowhead).



Figs 26–29. *Gelidium pteridifolium.*

Fig. 26. Fused gonimoblast cell and terminal nutritive cell following division of diploid nucleus. Arrowhead points to site of incomplete cytokinesis. A carposporangial initial (ca) has been cut off from an intercalary uninucleate gonimoblast cell.

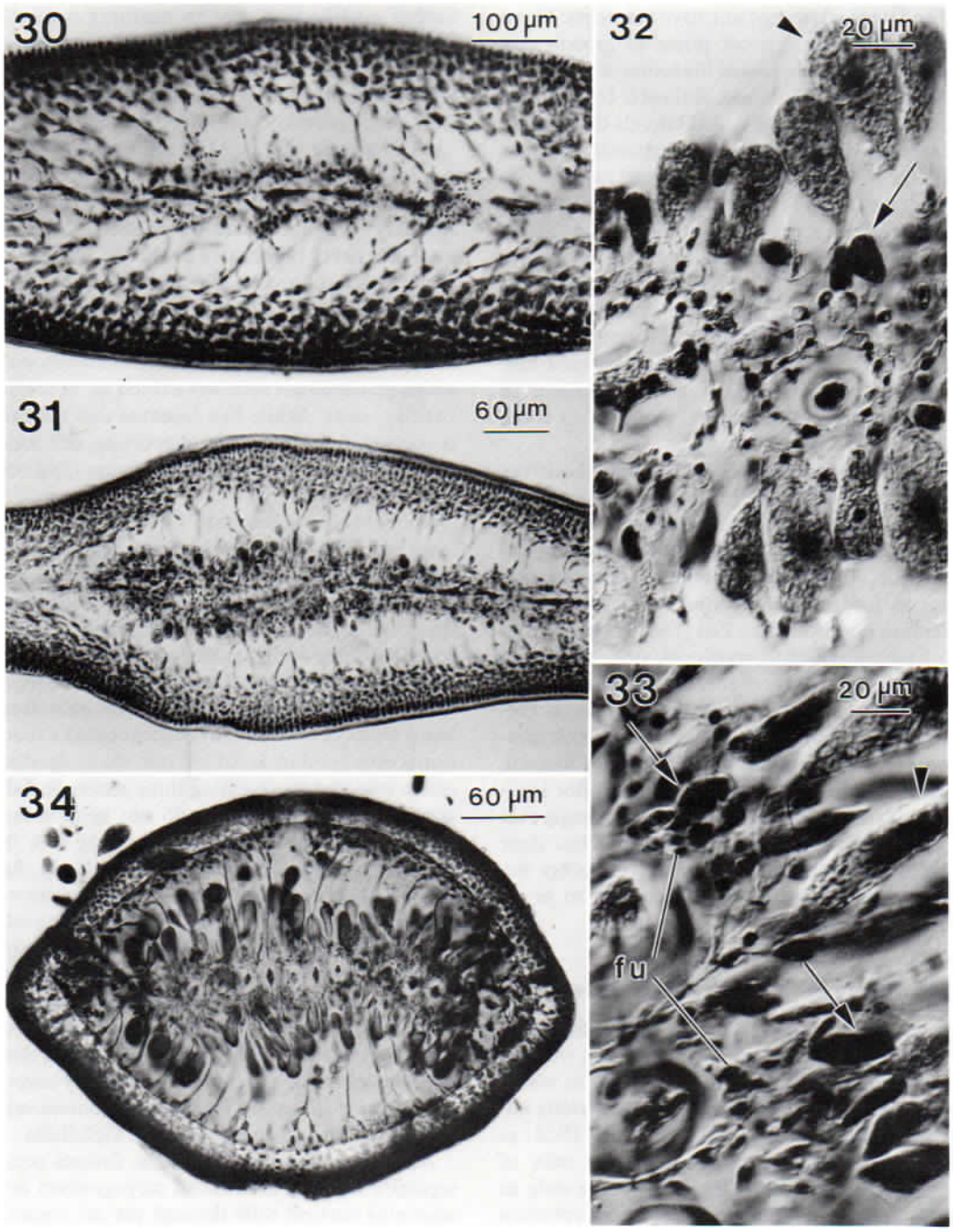
Fig. 27. Network of gonimoblast filaments (g) fused to terminal cells (fu) of nutritive filaments (nf) and bearing young carposporangia (ca).

Fig. 28. Network formed by secondary fusions of gonimoblast cells (arrowheads) onto fusion cells (fu) at tips of nutritive filaments.

Fig. 29. Squash preparation showing multinucleate fusion network at a later stage.

production of uninucleate gonimoblast initials. Such cells are especially large in *Suhria* and *Pterocladia* (Fan 1961, figs 7c, 11c).

In microscope preparations in which the cortex has been removed without disturbing the relationship between gonimoblast and nutritive filaments, it is evident that gonimoblast filaments branch in a regular fashion between the horizon-



Figs 30–34. *Gelidium pteridifolium.*

Fig. 30. Longitudinal section of young cystocarp with carposporangial initials and elongated inner cortical filaments.

Fig. 31. As in Fig. 30 at older stage.

Fig. 32. Cross-section of network of gonimoblast and nutritive filaments (now largely vacuolate) with mature primary (arrowhead) and immature secondary (arrow) carposporangia.

Fig. 33. Close up view of fusion network with mature primary carposporangia (arrowhead) and secondary carposporangia (arrow) from multinucleate fusion cells (fu).

Fig. 34. Median cross-section of cystocarp with mature carposporangia and opposite ostiolar regions.

tal and vertical rows of nutritive filaments. There appears to be a distinct phase of growth and branching of gonimoblast filaments in which the cells remain uninucleate, followed by the production of special one-celled laterals that extend toward cells of the nutritive filaments and fuse with them. Fan (1961) has illustrated fusions with both terminal and intercalary nutritive cells. The initial fusions involved only terminal nutritive cells or one-celled laterals and not intercalary cells in *Gelidium pteridifolium*. Intercalary nutritive cells become binucleate or multinucleate with the nuclei deeply staining. In agreement with Fan (1961) we find that gonimoblast cells fuse with intercalary cells of nutritive filaments in *Suhria* and *Pterocladia* and, perhaps, in some species of *Gelidium*.

In *Gelidium pteridifolium* the haploid nucleus of the terminal nutritive cell degenerates after fusion with a gonimoblast cell and the diploid nucleus divides, usually without completing cytokinesis. Additional gonimoblast cells then appear to fuse with the initial fusion cell which becomes multinucleate. Fan (1961) reported that both uninucleate and multinucleate gonimoblast cells cut off one to several carposporangia. We find that carposporangia are produced in two series; the first cut off by extant, uninucleate gonimoblast cells, and the second by later-formed, multinucleate fusion cells. It is only after initiation of the second series of carposporangia that unfused cells of the nutritive filaments lose their contents and become vacuolate. Pit plugs between adjacent nutritive cells appear to break down at this stage.

Are auxiliary cells present in Gelidiaceae?

As was noted earlier, Schmitz (1883) first used the term 'Auxiliarzellen' in conjunction with his observations on the 'Gelidieen' group in which the auxiliary cells appeared to have a strictly nutritive function. Kylin (1923, p. 132, 1928, p. 105) distinguished between auxiliary cells of Nemaliales and Gelidiales which serve only as nurse cells (= Nährzellen) and the 'typischen Auxilarzellen' of the higher orders of Florideophycidae that function as the starting point of gonimoblast formation. Papenfuss (1951) and Drew (1954) called the former 'nutritive auxiliary cells' and the latter, which have a dual function in that they furnish nutritive materials and also initiate the gonimoblasts, 'generative auxiliary cells'. Drew (1954, pp. 57, 65) added the further qualification that an auxiliary cell be a 'specified cell' of the gametophyte (occurring in specified position) with which the carpogonium fuses before formation of gonimoblasts or a cell with which primary gonimoblast fuses.

As Dixon (1982, p. 614) has remarked, the crucial difference of opinion between himself (Dixon 1959, 1961) and Fan (1961) as to whether auxiliary cells are absent or present in Gelidiaceae has never been stated explicitly. Dixon has restricted his discussion of possible auxiliary cells in Gelidiaceae to cells with which the fertilized carpogonium may have fused. Fan, on the other hand, treated cells of the nutritive filaments with which gonimoblast cells have fused as 'nutritive auxiliary cells'. While Fan reported that the carpogonium fuses with the supporting cell after fertilization, he did not call the supporting cell an auxiliary cell.

We heartily concur with Drew (1954) in her insistence that only cells that are 'specified' both with regard to their behavior and position can qualify as possible auxiliary cells. In Gelidiaceae the criteria that determine specificity must be tested both for cortical cells with which the carpogonium may fuse and for cells of the nutritive filaments with which the gonimoblast may fuse. Since we have not seen any evidence that a functional carpogonium is cut off from the supporting cell in material representing three genera and six species of Gelidiaceae, we do not believe that such a cell could serve as an auxiliary cell. By contrast, some of the drawings of Fan (1961, figs 6b, 9d) suggest that fusion may have occurred between the carpogonium and the supporting cell. Similar fusions have been reported in a species of *Beckerella* (Kraft 1976). This is a difficult point to prove, and many more observations will be needed before a final conclusion can be reached. We should add that it is not possible at this time to speculate as to whether an intercalary carpogonium or a one-celled lateral carpogonium represents the primitive condition in Gelidiales.

According to our observations, fusions occur regularly between a fertilized carpogonium and adjoining cortical cells through the pit connections. Only cortical cells distal to the carpogonium are involved and there is no evident modification of these cells prior to fertilization. The increase in the number and volume of nuclei in cortical cells distal to the carpogonium after fertilization appears to be associated with changes in the metabolic activity of these cells, perhaps due to modifications in levels of transcription

and translation of genetic information in the nuclear DNA. In some Gelidiaceae, particularly *Suhria*, the cortical cells fuse forming large, lobed fusion cells which, however, never function directly in gonimoblast formation. There seems to be a high degree of variation in Gelidiaceae with regard to the level of involvement of the cortical cells in the nutrition of a developing carpogonium. Until their role is clarified for a large number of species, we feel that such cells should be regarded as ordinary nutritive cells and not as nutritive auxiliary cells.

In contrast to the cortical filaments, the nutritive filaments are preformed and specially adapted for a nutritive function prior to fertilization. Following fusion with gonimoblast cells their nuclei may divide and additional fusions may occur, but ultimately cells of the nutritive filaments become vacuolate as their contents are consumed. In *Pterocladia* and *Suhria* gonimoblast filaments fuse almost at random with both terminal and intercalary nutritive cells. There is no indication that any particular fusions are specified and nutritive cells appear to be contacted separately and utilized individually, or they may be incorporated into a dendroid fusion cell (Fan 1961, pl. 43). In *Gelidium pteridifolium*, on the other hand, the terminal cells are distinguishable from other cells of the nutritive filaments and appear to be specialized for contact and fusion with terminal gonimoblast cells. This latter situation represents an evolutionarily advanced condition approaching that expected for an auxiliary cell; however, by comparison with auxiliary cells of other orders of red algae the terminal cells of nutritive filaments are not highly differentiated and their position is not well defined. We would conclude then with Dixon (1961) that auxiliary cells are absent in Gelidiaceae, but that particular fusions occurring in this family represent an early evolutionary stage that could potentially give rise to an independent auxiliary cell system.

Characterization of the order Gelidiales

The following description is based on J. Agardh (1876), Kylin (1928), Dixon (1958, 1959), Fan (1961), Chihara & Kamura (1963), Kaneko (1966, 1968), McCandless (1981), Pueschel & Cole (1982), Yaphe (1984), Carter (1985), Maggs & Guiry (1987) and our observations.

The Gelidiales Kylin (1923) are characterized by a *Polysiphonia*-type life history in which male, female and tetrasporangial or bisporangial plants are nearly identical morphologically. Sex and phase ratios are variable with tetrasporophytes often predominating, or tetrasporophytes may be the only known phase. Cell walls and the intercellular matrix contain agar and other polymers characteristic of agarophytes. Pit connections have pit plugs with a single cap layer. Early development of carpospores and tetraspores is identical and follows a prescribed pattern. Following nuclear division, most of the spore contents pass into the germ tube leaving a largely evacuated spore which is then cut off. The germling initial divides obliquely by a curved wall into cells of unequal size. One, two or more rhizoids are initiated from the distal end, and an apical initial is subsequently formed from the proximal end near the spore wall. Vegetative growth takes place by transverse division of an apical cell producing a uniaxial thallus. Each axial cell bears two opposite periaxial cells that produce lateral filaments of the second order. These are normally interconnected longitudinally by secondary pit connections. Additional secondary pit connections between cortical or medullary cells are common. Filaments of the third order arise in opposite pairs from cell rows of the second order and grow perpendicular to a plane passing through the second-order filaments and the central axis. Thick-walled internal rhizoids develop from cells of third- and higher order cortical filaments and grow basipetally, descending through the cortex and/or medulla, or internal rhizoids are present only at attachment points or are absent. Branching is initiated through the transformation of surface cells of filaments of limited growth into apical initials of filaments of unlimited growth.

Sexual plants are dioecious, or rarely monoecious. Male fertile areas form irregular colorless patches or small sori on the surface of the fertile branchlets. Surface cortical cells elongate and divide anticlinally to produce the spermatangial initials. Spermatangia are formed beneath the surface membrane by transverse division of the initials.

The female reproductive system develops near the apex on lateral or superficial pinnules, or on ordinary branches, and consists of carpogonia and nutritive filaments before fertilization. The nutritive filaments are branched, short-celled filaments that develop primarily from the basal cells of vegetative filaments of the third order in the fertile area, or sometimes on higher orders

and translation of genetic information in the nuclear DNA. In some Gelidiaceae, particularly *Suhria*, the cortical cells fuse forming large, lobed fusion cells which, however, never function directly in gonimoblast formation. There seems to be a high degree of variation in Gelidiaceae with regard to the level of involvement of the cortical cells in the nutrition of a developing carpogonium. Until their role is clarified for a large number of species, we feel that such cells should be regarded as ordinary nutritive cells and not as nutritive auxiliary cells.

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of filaments. Functional carpogonia are intercalary and usually develop from suprabasal cells of third-order filaments. They normally occur along the sides of the central furrow in a fertile area. Commonly, carpogonia are cut off as sessile laterals that appear to be non-functional. A fertilized carpogonium enlarges and becomes multinucleate as it forms processes that cut off uninucleate gonimoblast initials. Often multinucleate cells are cut off that produce additional uninucleate gonimoblast filaments. Cortical filaments borne on the carpogonium become modified, their nuclei divide and enlarge, and channels develop through the pit connections leading to cytoplasmic continuity with the fertilized carpogonium. The modified cortical cells either remain distinct or form lobed fusion cells. Gonimoblast filaments grow and ramify between the files and rows of nutritive filaments producing laterals that fuse with terminal or intercalary cells of the nutritive filaments. Additional gonimoblast cells may unite with the resulting fusion cells. Carposporangia are cut off from uninucleate gonimoblast cells or from both uninucleate gonimoblast cells and multinucleate fusion cells. Cystocarps are raised, oval to circular in outline and either unilocular with one potential ostiole, or bilocular with a central partition and two potential ostioles. Carposporangia are clavate to obovate and are borne either in chains on a basal placenta or singly and either solitarily or in terminal clusters on paired placentae alongside the central partition. Inner sterile cortical cells elongate during cystocarp enlargement to form filaments that extend through the cavity of the cystocarp from the base or central partition to the pericarp.

Asexual reproduction is by tetrasporangia or bisporangia. Sporangial initials are cut off laterally from the apical cells of cortical filaments by anticlinal division. At maturity they are laterally pit connected and become embedded as a result of the continued growth of surrounding cortical filaments. Tetrasporangia are broadly ellipsoid to spherical in shape and cruciately or tetrahedrally? divided into four spores each with one nucleus. Bisporangia are transversely divided into two spores, each most commonly containing two nuclei.

Phylogenetic relationships of the Gelidiales

The Gelidiales contain either a single family or two families: Gelidiaceae and Gelidiellaceae. Fan (1961) erected the Gelidiellaceae to include a single genus, *Gelidiella*, that lacked internal rhizoids in the vegetative thalli and in which a sexual phase was unknown. Recently, Maggs and Guiry (1988) have reported the presence of medullary rhizoids near the attachment points in a species of *Gelidiella* and have concluded that Gelidiellaceae should be merged with Gelidiaceae.

Kylin (1928, p. 115) commented that the Gelidiales were not closely related to any other orders of Florideae. Fan (1961) suggested a possible relationship of the Gelidiales with the Nemaliales through the Naccariaceae and with the Cryptonemiales through *Acrosymphyton*. Dixon (1961) treated the Gelidiaceae as a coherent family within a broadly defined order Nemaliales pending complete revision of the classification of the Florideae.

The special nutritive filaments in the Naccariaceae mentioned by Fan (1961) are borne on the carpogonial branch and are not related developmentally to the nutritive filaments of Gelidiales. A carpogonial branch is present in Naccariaceae but absent in Gelidiales and the fusion of the carpogonium with cells of the carpogonial branch or the supporting cell in Naccariaceae is not equivalent to fusions of the carpogonium with cortical cells in Gelidiales. The auxiliary cell branch in *Acrosymphyton* is homologous to the highly modified carpogonial branch in that genus. Its similarity to the nutritive filaments of Gelidiales reflects the fact that both structures have a similar nutritive function.

Members of the Gelidiales possess pit plugs having only one cap layer (Pueschel & Cole 1982), a feature that serves to separate them from the Nemaliales in which pit plugs have two cap layers, and presumably also from most higher Florideophycidae in which plug caps are absent. We conclude with Kylin (1928) that the Gelidiales possess a combination of diagnostic characters that sets them apart from all other orders presently recognized among the red algae. Close scrutiny of the developmental basis for each of the vegetative and reproductive characters that are recognized in Gelidiales suggests that each had an independent origin and evolutionary history, separate from comparable structures seen in other assemblages of red algae. Virtually the only developmental similarities involve characters that are common to all orders of Florideophycidae. We therefore conclude that the Gelidiales is a natural order that is related to other orders of Florideophycideae only through an ancestor

common to all of them, and that the inclusion of the Gelidiaceae as a family within a broadly defined order Nemaliales as proposed by Dixon (1961, 1982) is no longer tenable.

At the present time only the most tenuous biochemical, cytological and anatomical characters hint at possible special phylogenetic relationships of the Gelidiales within the Florideophycidae. In this connection, some similarities between the Gelidiaceae and the Gracilariaceae may prove interesting. Both groups are agarophytes in which agarobiose (3,6-anhydro-4-O-β-D-galactopyranosyl-L-galactose) is the repeating unit, and both appear to show comparable variations in the levels of neutral agarose and agarose molecules substituted with sulfate and pyruvate groups in different species (Yaphe 1984). Although *Gelidium* is reported to have a single cap layer and *Gracilaria* is said to have none (Pueschel & Cole 1982), a cap does appear to be present on the side of the host cell in pit connections between *Holmsella* and *Gracilaria* (Wetherbee & Quirk 1982b) and striations can occur perpendicular to the ends of the core in *Gracilaria* (Wetherbee & Quirk 1982a) that appear similar to those seen in *Gelidium* (Pueschel & Cole 1982). The behavior of the spermatangial initials in cutting off spermatangia by transverse divisions is recorded in both Gelidiaceae and Gracilariaceae. In *Gracilaria* the carpogonium is terminal, whereas in *Gelidium* it is intercalary; nonetheless, the carpogonium enlarges and becomes multinucleate after fertilization in both groups either prior to or during gonimoblast initiation. Typical auxiliary cells appear to be absent in both groups (pers. obs.).

While the carrageenophytes may all be members of the Gigartinales (*sensu* Kraft & Robins (1985)), the agarophytes range over a wide assemblage of seemingly unrelated orders and families. Perhaps the agarophytes are evolutionarily the older group and the Gelidiaceae and Gracilariaceae are related members of that broad assemblage. The suggestion originally made by Stoloff & Silva (1957) that water soluble polysaccharides may be significant for the classification of red algae at higher taxonomic levels seems particularly relevant to the present time.

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