S. Fredericq.

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Developmental morphology of *Gigartina pistillata* (Gigartinaceae, Rhodophyta)

M. HOMMERSAND[1], S. FREDERICQ[1] AND J. CABIOCH[2]

[1]*Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, USA*
[2]*Station Biologique, Place Georges Teissier, 29682 Roscoff Cédex, France*

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The vegetative and reproductive development of the type species of the marine red alga *Gigartina*, *G. pistillata* (S.G. Gmelin) Stackhouse (Gigartinaceae, Rhodophyta), is described based on material from Brittany, France. *Gigartina pistillata* possesses a loose primary network of medullary filaments in which every cell is normally connected to each of its neighbouring cells by a single primary or secondary pit connection. Secondary filaments up to three cells long fill the gaps created as the network expands and link terminally to cells below by secondary pit connections. The procarp is derived by successive concavo-convex divisions of a surface cell that is homologous to the apical initial of a vegetative cortical filament, and consists of an inner cortical supporting cell bearing a curved, 3-celled carpogonial branch and a single, lateral cortical filament. After presumed fertilization, the carpogonium fuses with the auxiliary cell effecting transfer of the zygotic nucleus. Small diploid nuclei increase in number and become intermixed with larger haploid nuclei in the cytoplasm of the auxiliary cell. An enveloping tissue composed of short, branched filaments is produced from vegetative cells around the auxiliary cell. Gonimoblast filaments penetrate this tissue, and gonimoblast and vegetative cells fuse or unite by secondary pit connections to form a heterokaryotic placenta. Carposporangial chains derived from both diploid gonimoblast and heterokaryotic placental cells differentiate in clusters, separated by sterile placental tissue and surrounded at the periphery by the enveloping tissue. Tetrasporangial sori are formed progressively at the boundary between cortex and medulla. Cruciately divided tetrasporangia are intercalary and originate from uninucleate cells in both primary and secondary filaments. Sporangia in each tetrasporangial sorus are evidently released as a unit at maturity, leaving an exposed surface which is repaired by means of the proliferation of subcortical cells.

INTRODUCTION

Recent studies strongly suggest that four families in the Gigartinales (Chondriellaceae, Phyllophoraceae, Petrocelidaceae and Gigartinaceae) form a closely related complex characterized by the universal presence of the sulphated galactan carrageenan, multiaxial growth, abundant secondary pit connections, curved, 3-celled carpogonial branches, procarps in which the supporting cell functions as an auxiliary cell bearing gonimoblasts that arise severally and develop primarily inwardly, and cruciately divided tetrasporangia (Guiry *et al.* 1984; Guiry & Garbary 1990; Hommersand 1990). The systematic position of many of the species and genera within each family, and the taxonomic status of the family complex in relation to the rest of the Gigartinales, are problems that need to be resolved. Observations in culture of species belonging to *Gigartina* subgenus *Mastocarpus* established the presence of a heteromorphic life history with tetrasporangia borne singly in an intercalary position in the crustose phase, and led to reinstatement of the genus *Mastocarpus* Kützing (1843) and its transfer to the Petrocelidaceae (Guiry *et al.* 1984). Morphological studies have, however, been few, and, except for the excellent descriptions and illustrations of the Japanese species of Phyllophoraceae and Gigartinaceae by Mikami (1965), have provided little new information. A major revision of the Gigartinaceae was attempted by Kim (1976), who accepted two genera: *Chondrus*, for a small number of species from the North Atlantic and western North Pacific Oceans, and *Gigartina*, to which he transferred all the remaining species of the other genera recognized by J. Agardh (1851, 1876), Kylin (1956) and Mikami (1965). Although Kim's study was interesting and informative, his conclusions have not met with general acceptance. At present, the tax-

onomy of this large, economically important family remains confused.

As an initial contribution towards clarifying the generic relationships within the Gigartinaceae we here document the vegetative and reproductive morphology of *Gigartina pistillata* (S.G. Gmelin) Stackhouse, the type species of *Gigartina*.

MATERIALS AND METHODS

Plants were fixed in 8–10% Formalin/seawater for at least 24 h and preserved in 5% Formalin/seawater. Best results were obtained when the material was fixed in the field within 30 min of collection. Material selected for examination was placed under a 40 W tungsten lamp in 5–10% Formalin/seawater for at least a week prior to sectioning, or until bleached completely white. Better results were obtained with 12-month-old collections as opposed to freshly preserved material, and even older collections were usually satisfactory. Thallus pieces were rinsed briefly with distilled water to remove salt crystals before sectioning. Periclinal, longitudinal and transverse sections were made by hand with a platinum-chrome double-edged razor blade. For periclinal sections, thallus strips not exceeding 0.5 cm in length or width were held between forceps and split open once or twice, depending on the thickness of the sample. Pieces intended for longitudinal or transverse sections were first air-dried or placed on a hot microscope lamp-housing for a short time (< 1 min) until optimal firmness was attained, and held on a microscope slide with an index finger or with forceps and hand-sectioned. Most sections were stained with aceto-iron-haematoxylin-chloral hydrate (Wittmann 1965) for periods ranging from 30 min to several hours. If a few large sections were to be stained, they were re-expanded briefly on the slide in distilled water, after which the water was removed with tissue paper, 2–3 drops of haematoxylin stain pipetted onto the sections, and a coverslip applied. If the sections were small and numerous, distilled water was added, a coverslip applied, and several drops of haematoxylin stain were flushed underneath the coverslip from one side and drained off from the other with tissue paper. If needed, a coverslip was placed over the dry sections and distilled water and haematoxylin stain added and removed successively under the coverslip to prevent the sections from curling. Slides were destained with 45% acetic acid solution, either by removing the coverslip or by adding solution from one end of the slide and draining from the other. In general, thick sections were stained and destained for longer than thin sections. When appropriate differentiation of nuclei or tissues was obtained, as observed under a compound microscope, one drop of 1 : 1 Hoyer's mounting medium : water (Stevens 1981) was added along one edge and either drained from the other, or the coverslip was lifted off, the excess stain removed with tissue paper, a drop of 1 : 1 Hoyer's : water medium placed directly on the section, and a new coverslip applied. With thin sections, satisfactory destaining was adjudged to have been achieved when nuclei and pit plugs just began to differentiate compared with background cytoplasm. Sections of vegetative thalli were sometimes stained with 1% aniline blue and mounted in 50% Karo® corn syrup. Bright field pictures were taken with a Zeiss Photomicroscope III using PAN-X or T-MAX Kodak film. Pictures were taken at optimum times during the staining process or after transfer to the Hoyer's medium, up to 3 d, depending on the rate of differentiation of nuclei, pit plugs and cytoplasm in the stain or mounting medium. Hoyer's medium was used for stain differentiation and not to prepare permanent slides.

Herbarium abbreviations follow Holmgren *et al.* (1990).

OBSERVATIONS

Gigartina Stackhouse (1809, p. 55)

NOMENCLATURAL HISTORY: Stackhouse (1809, p. 55) erected the genus *Gigartina* for a single species, *Gigartina pistillata* based on *Fucus pistillatus* S.G. Gmelin (1768, p. 159). Turner (1802, p. 280) was the first to recognize the identity of *Fucus gigartinus* Linnaeus (1759, p. 1344) and *Fucus pistillatus*, an opinion followed by Lamouroux (1805, p. 51). Interestingly, Stackhouse (1816, pp. x, xii, 62) subsequently questioned this identity. Although *Fucus gigartinus* has temporal priority over *F. pistillatus*, this epithet cannot be used as it would result in a tautonymic combination (see Art 23.4, International Code of Botanical Nomenclature; Greuter *et al.* 1988). As discussed by Setchell & Gardner (1933, p. 255) *Gigartina* was incorrectly attributed to Lamouroux (1813, p. 134) for most of the 19th

century, often as emended by J. Agardh (1842, p. 103).

ETYMOLOGY: From the Greek *gigarton,* grape stone or drupe; refers to the drupe-like appearance of the cystocarp.

TYPE SPECIES: *Gigartina pistillata* (S.G. Gmelin) Stackhouse.

Gigartina pistillata (S.G. Gmelin) Stackhouse (1809, p. 74)

BASIONYM: *Fucus pistillatus* S.G. Gmelin (1768, p. 159).

LECTOTYPE: Original illustration in the absence of material (S.G. Gmelin 1768, pl. 18, fig. 1).

TYPE LOCALITY: Doubtful, sent by D.D. Sandifort from The Hague, The Netherlands.

SYNONYMS: *Fucus gigartinus* Linnaeus (1759, p. 1344); ?*Fucus oederi* Esper (1802, p. 52); *Ceramium gigartinum* (Linnaeus) Roth (1806, p. 409); *Sphaerococcus gigartinus* (Linnaeus) C. Agardh (1823, p. 274).

SPECIMENS EXAMINED: Ile verte, Roscoff, Brittany, France (*Cabioch,* cystocarpic, 18.ii.1991, 19.iii.1991, 20.iii.1991, 11.ix.1991; male, cystocarpic, 12.vi.1991, NCU); Santec, Brittany, France (*Cabioch & Garbary,* male, cystocarpic, 25.vi.90; *Cabioch,* tetrasporangial, 19.iii.1991, cystocarpic, 18.iv.1991, 15.v.1991; *Cabioch & Hommersand,* cystocarpic, 15.vi.1991; *Cabioch,* cystocarpic, 14.vii.1991, 12.viii.1991, 10.ix.1991, tetrasporangial, 12.viii.1991, NCU).

DISTRIBUTION: Eastern Atlantic (southern Ireland south to Mauritania), ?South Africa.

HABITAT: Intertidal to upper sublittoral, epilithic in pools and occasionally emergent; in sheltered areas and some areas exposed to wave action; tolerant of sand cover (Feldmann 1954; Dixon & Irvine 1977).

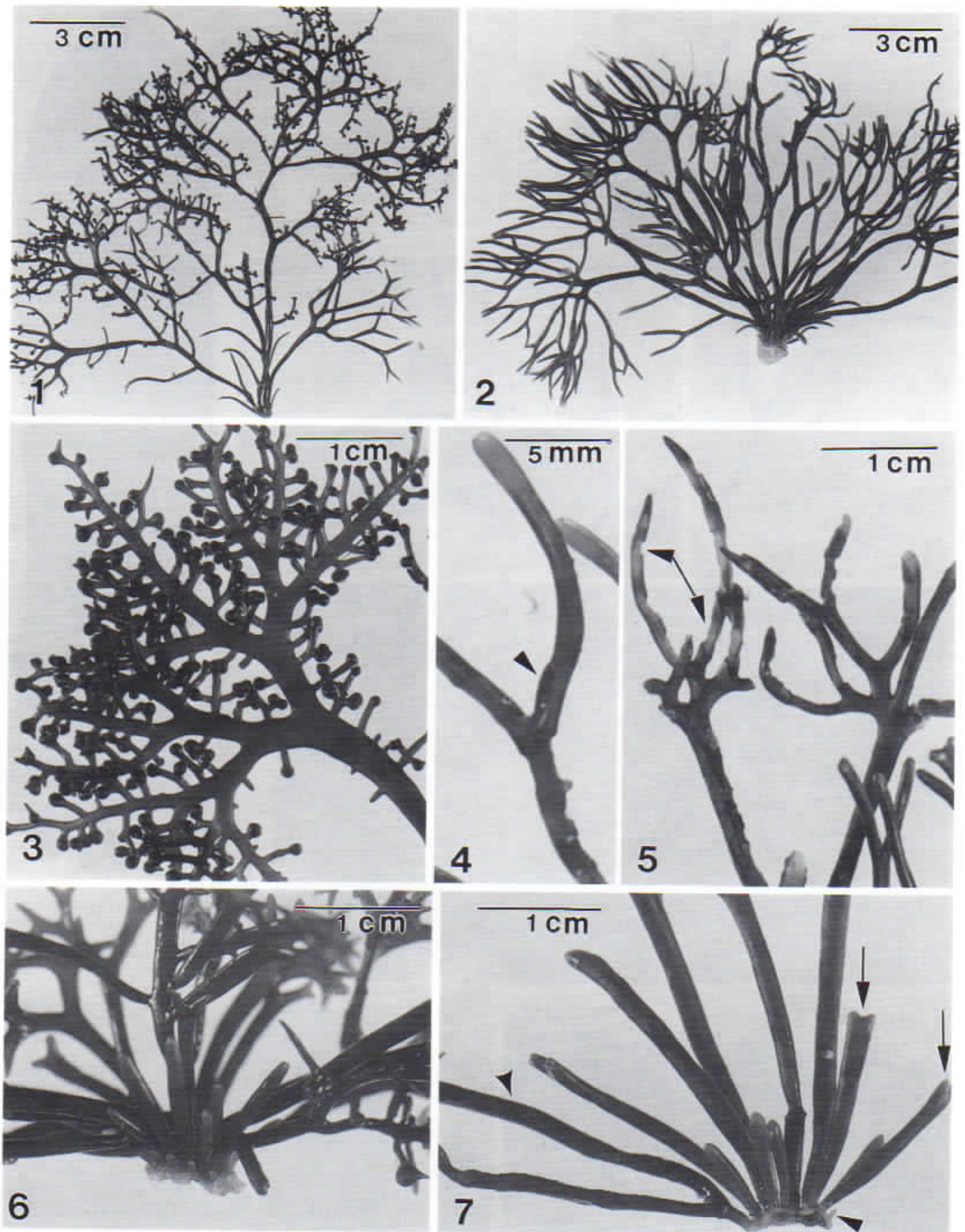
HABIT: *Gigartina pistillata* is a rather common species at low tide levels in the vicinity of Roscoff, Brittany, occurring in shaded habitats protected from heavy wave action (Feldmann 1954). The species can withstand extensive, nonpermanent sand covering. It is particularly abundant at Santec (5 km west of Roscoff) where it occurs primarily in isolated clumps. Adult plants 7–15(–20) cm high are present throughout the year. Clumps are generally sterile but some are always cystocarpic (Figs 1, 3). Tetrasporangial plants are less frequent (Figs 2, 4–5). A single mature tetrasporangial plant bearing a side branch with cystocarps was collected in March, 1991. Unbranched, upright juvenile thalli arise from discoid or crustose holdfasts in spring and early summer. Some come from single discs less than 1.5 mm in diameter and appear to be under a year old. Discs this size are often fused laterally or overlie other discs or crusts. Larger crusts 10 mm or more in diameter are probably several years old. They conform to the topography of the substratum and are irregular in shape. During spring and early summer, crustose holdfasts support a mixture of juvenile and adult thalli (Figs 1–2, 6–7).

Juvenile thalli are straight, unbranched, cylindrical, constricted at the base, and nearly uniform in diameter above the basal 1.0–1.5 mm (Figs 6–7). The first dichotomy is initiated at a height of 1–2(–4) cm (Fig. 7). Branches occurring lower down on the plants are usually adventitious in origin (Fig. 7). Adult plants are 5–8(–12) times dichotomously branched and subcylindrical to compressed, ranging from 1.5–4 mm broad × 1.5–2 mm thick.

Female gametangial plants bear subopposite pinnules 0.5–2 cm long, produced adventitiously from the margins of main branches (Figs 1, 3). The first pinnules typically originate between dichotomies in the mid-region of the thallus and later extend towards the tips and base, although the stipe usually remains non-proliferous. Pinnules are simple to once or twice compound in female plants with the cystocarps subterminal on unbranched pinnules, or briefly stalked and often opposite on branched pinnules (Figs 1, 3).

Tetrasporangial plants are less congested than gametangial plants and usually bear simple pinnules, or pinnules may be absent (Fig. 2). Tetrasporangial sori form raised elliptical to linear patches on main branches and pinnules (Figs 4–5). An entire sorus is released at one time leaving an eroded surface exposing the medulla (Fig. 5). As the surface is repaired by proliferation of subcortical cells, new sori are generated in adjacent cortical areas and the process is repeated until virtually all the original cortex above the stipe has been converted and sloughed off.

HOLDFAST AND JUVENILE UPRIGHTS: Some young plants, those presumably derived from sporelings, consisted of a single erect thallus attached by a discoid holdfast up to 1.5 mm in diameter (Figs 8–9). Older plants contained a mixture of mature and juvenile erect thalli attached by a larger crustose holdfast. A longitudinal section through a holdfast reveals a core of isodiametric and vertically elongated cells directly below the erect axis surrounded by a ring of radiating hor-



Figs 1-7. *Gigartina pistillata.* Habits (NCU).

Fig. 1. Habit, cystocarpic plant (*Cabioch,* 18.ii.1991).

Fig. 2. Habit, tetrasporangial plant (*Cabioch,* 19.iii.1991).

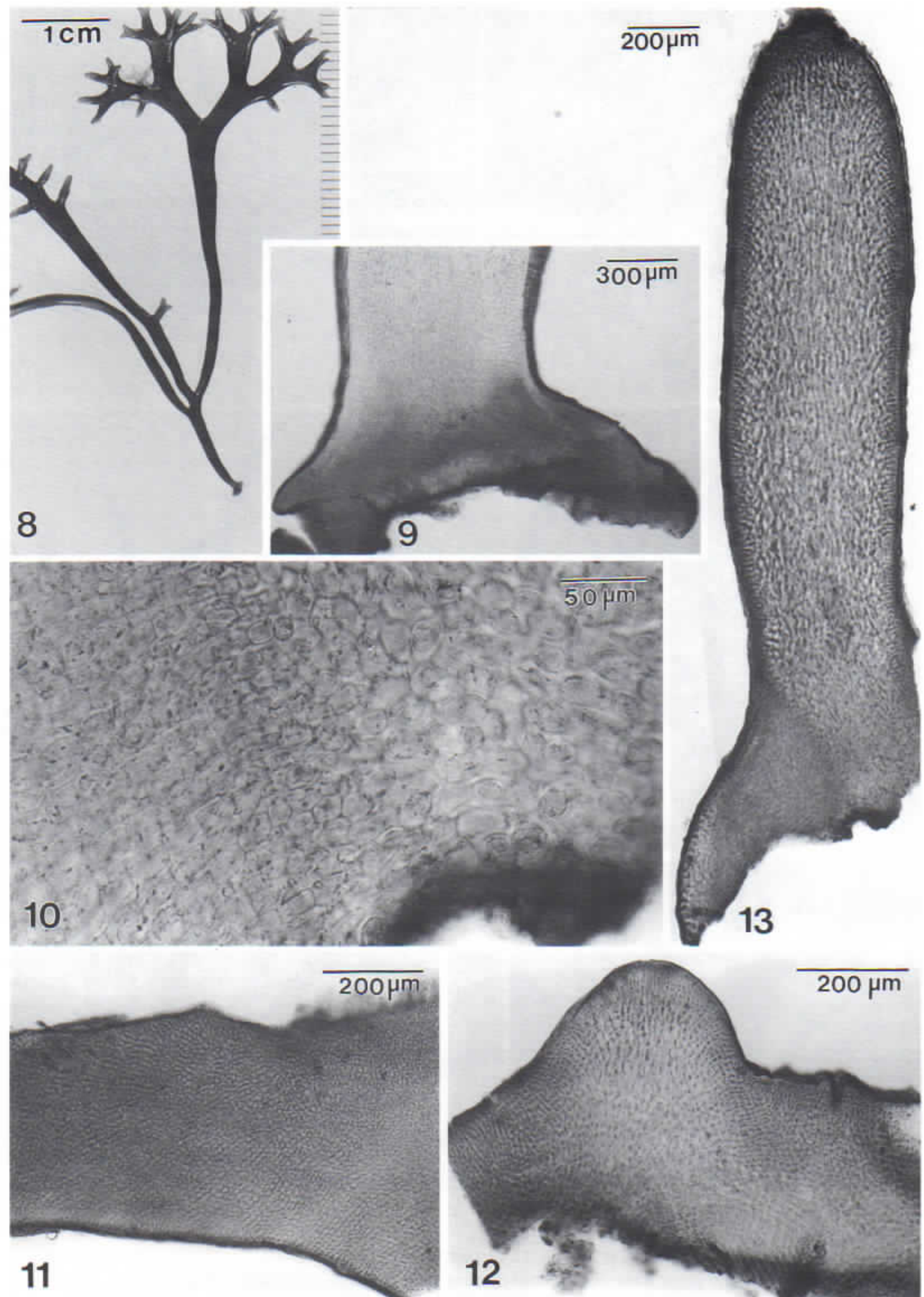
Fig. 3. Branch of female plant with subterminal cystocarps on pinnules (*Cabioch,* 19.iii.1991).

Fig. 4. Raised tetrasporangial sorus (arrowhead) (*Cabioch,* 19.iii.1991).

Fig. 5. Eroded areas (arrows) following tetrasporangial sorus release (*Cabioch,* 19.iii.1991).

Fig. 6. Crustose holdfast bearing juvenile uprights along margin and young adult thalli (*Cabioch & Hommersand,* 15.vi.1991).

Fig. 7. Crustose holdfast bearing cylindrical juvenile uprights (straight arrowhead). First dichotomies (arrows) and an adventitious lateral borne on an old branch (curved arrowhead) are indicated (*Cabioch & Hommersand,* 15.vi.1991).



Figs 8-13. *Gigartina pistillata.* Habit and morphology of basal system (*Cabioch & Garbary,* 25.vi.1990, NCU).
Fig. 8. Habit of young adult plant with discoid holdfast and 7–8 orders of dichotomous branches.
Fig. 9. Enlarged view of discoid holdfast (aniline blue).
Fig. 10. Boundary between filaments at base of upright (right) and horizontal filaments of crust (left).
Fig. 11. Section through crust showing regular patterns of filament radiation (aniline blue).
Fig. 12. Section through crust showing initiation of dome-shaped upright (aniline blue).
Fig. 13. Longitudinal section through juvenile upright.

izontal filaments. The boundary between the two regions is sometimes abrupt (Fig. 10), and horizontal filaments in the holdfast tend to merge with cortical filaments further along the stipe.

Growth of the holdfast is maintained through the activity of a marginal meristem. Crustose holdfasts are variable in thickness and indefinite in outline, and are composed of horizontal filaments and filaments that arch towards the substratum or the external surface in patterned arrays (Figs 11–12). The layer underlying the holdfast is about the same thickness as the outer cuticle (10–20 μm), and stains to about the same extent with haematoxylin or aniline blue (Figs 9–13). Filaments radiating towards the surface tend to be uniform in diameter and close fitting with little intervening space (Figs 11–12).

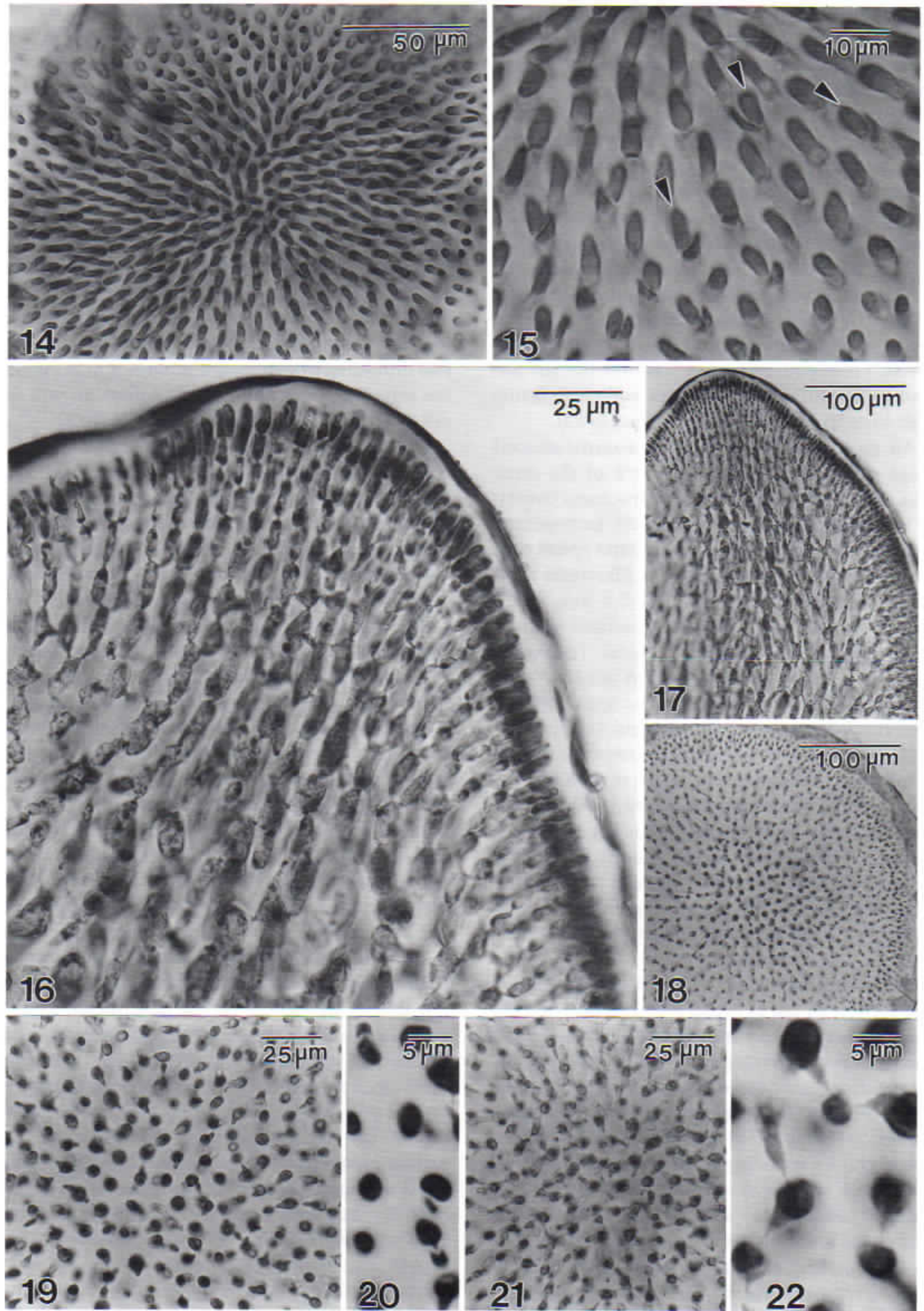
An erect axis is initiated as a dome-shaped bulge welling up from the surface of the crust (Fig. 12). These dome-shaped structures consist of vertically elongated crustal cells surmounted by a cluster of locally dedifferentiated apical cells and their derivatives. Figure 13 illustrates a juvenile upright 2.5 mm tall and 0.5 mm in diameter as seen in median longitudinal section. Growth in length and width is apical. The cortex becomes constant in thickness at about 50 μm approximately 0.5 mm below the apex, and the medullary filaments cease elongation. The medulla is exceptionally compact, composed largely of longitudinal filaments arranged in parallel. The principal difference between an erect juvenile axis and a branch of an adult thallus relates to the degree of separation of the filaments and the amount of space occupied by intercellular matrix. Juvenile uprights do not expand laterally as rapidly as adult thalli, and their medullary filaments are more compact.

Apical cells at the tip of a juvenile thallus are about twice as tall as broad after cell division (7–8 μm by 3–4 μm). An apical cell doubles in length and divides transversely, usually followed by longitudinal, concavo-convex division of the terminal cell (Fig. 16). The subterminal cell enlarges alongside the terminal cell and cuts off a second apical cell by an oblique, concavo-convex division (Figs 15–16). A common pattern is for a pair of longitudinal divisions to follow each transverse division, with the result that there is an unbranched segment between each branch-bearing segment (Fig. 16). In rapidly growing upright juvenile thalli, successive transverse divisions may occur between longitudinal divisions resulting in 2–3 unbranched segments between branch-bearing segments.

In juvenile thalli, the first apical cell cut off by a longitudinal division usually faces towards the centre of the apex (Figs 14–15). Each inwardly facing apical cell functions as the initial of a longitudinal filament, whereas the apical cell cut off from a subterminal cell initiates a radiating filament (Figs 15–16). Longitudinal filaments directly below the apical dome form a core in the centre of the medulla that is initially distinct from the surrounding cylinder of radiating filaments composing the outer medulla and cortex (Figs 17–18). Thus, apical growth of juvenile thalli is multiaxial and monopodial, consisting of a central core of longitudinal filaments and a fountain-like unfolding of radiating filaments which forms the outer medulla and cortex. Apical growth is not as regular in adult as in juvenile thalli.

One or two segments below the apex, intercalary cells cut off conjunctor cells laterally which fuse with neighbouring cells and form secondary pit connections. A conjunctor cell is initiated as a bulge in the middle or towards the base of an intercalary cell 2–3 segments below the apex (Fig. 16). The conjunctor cell is cut off laterally together with its nucleus and fuses horizontally with a neighbouring intercalary cell. The orientation of conjunctor cells and secondary pit connections is best seen in serial cross sections immediately behind the apex (Figs 19–22). A donor cell of a secondary pit connection sits adjacent to the incipient pit plug (Figs 19–20), whereas the recipient cell possesses a lateral extension formed by the flow of cytoplasm into the fused conjunctor cell (Figs 21–22). The number of nuclei in a recipient cell increases with each new secondary pit connection; however, most medullary and inner cortical cells contain more nuclei than the number of pit connections linking them to adjoining cells, suggesting that nuclear divisions are not strictly tied to the production of conjunctor cells. In practice, most laterally contiguous cells are linked by secondary pit connections. We have never seen two pit connections between the same pair of cells. Cells not initially connected by secondary pit connections immediately below the apex subsequently become linked, usually within 8–10 segments behind the tip (Figs 16–17). Apparently, any omissions that might leave a gap between neighbouring cells are ultimately corrected with formation of a secondary pit connection. Other details in the development of juvenile uprights are similar to those of the adult thallus.

ADULT THALLUS: Adult thalli branch dichotomously (Figs 1–3, 6, 8, 23). Some individuals,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especially tetrasporangial plants, are terete, but most are compressed to varying degrees in the plane of the branches. Compressed forms have broad apices. Nevertheless, apical cells converge towards the centre of the apical dome and also produce a central core of medullary filaments as in juvenile erect axes (Figs 23–24). Individual cells enlarge and the network of primary filaments expands close to the apex in adult thalli as matrix material is secreted into the intercellular spaces. Medullary cells are broadly stellate, as seen in a median periclinal section (Fig. 23), and are relatively narrower when viewed in a longitudinal section perpendicular to the direction of thallus compression (Fig. 24). Secondary filaments 1–3 cells long are produced in abundance beginning 10–12 segments below the apex (Figs 23–24). Most grow obliquely downwards and connect by means of terminal conjunctor cells to nearby cells below.

The growing region extends over the entire apex in an adult plant. Radiating filaments continue to grow and branch long after a recognizable cortex has formed. Outer cortical cells cut off conjunctor cells and form secondary pit connections up to 1 mm behind the apex (Fig. 25), whereas inner cortical cells produce 1- to 3-celled filaments that link terminally to nearby cells as long as the thallus is expanding and extending in length (Figs 26–27). The width of the cortex remains constant at 6–7 cells or about 50–60 μm, even as thallus expansion takes place. Outer cortex becomes inner cortex with the formation of secondary pit connections and inner cortex is transformed progressively into medulla as short secondary filaments are formed (Figs 23–25). While still within the growing region, primary and secondary medullary cells initiate additional filaments that extend through the intercellular matrix and connect to neighbouring cells in a process that is repeated as long as the thallus is expanding (Figs 26–27).

Once thallus expansion has ceased, cells of primary and secondary filaments become inflated and new filaments are no longer produced (Figs 28–30). In young, actively growing thalli, cell maturation usually takes place within 1 mm of the thallus apex. Individual cells increase to 2–3 times their original diameter, and the space between them is greatly reduced. Figures 28–30 illustrate a transitional stage in which the inner medullary cells have matured but outer medullary and cortical cells are still growing. Mature, inflated medullary cells have nearly the same diameter in periclinal and longitudinal sections (Figs 29–30). Except for the stipe and terminal branches, mature cortical and medullary tissues of young thalli are similar in appearance throughout and are composed of inflated cells.

The stipe represents the original juvenile erect axis and is distinct anatomically from the branches above. Numerous rhizoidal filaments are produced as the stipe expands secondarily and often becomes compressed. Irregularities occur in the extended cortex (Fig. 31). Large, stellate primary cells in the medulla are distended laterally as seen in periclinal section, and are surrounded by numerous secondary and rhizoidal filaments (Fig. 32). All are narrower in longitudinal section (Fig. 33). The resulting tissue is compact, with filaments filling in most of the intercellular space.

ADVENTITIOUS BRANCHLETS (PINNULES): Subopposite pinnules 0.5–2(–4) mm long, found particularly on fertile female plants, arise adventitiously through resumed growth of cortical tissue

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Fig. 14-22. *Gigartina pistillata.* Development of juvenile upright (*Cabioch & Garbary,* 25.vi.1990, NCU).

Fig. 14. Surface view of apex showing filaments converging towards tip (aniline blue).

Fig. 15. Enlarged view of lower right-hand part of apex in Fig. 14, showing apical cells of converging adaxial filaments (arrowheads) and of radiating abaxial filaments (aniline blue).

Fig. 16. Longitudinal section of tip showing transversely and longitudinally divided apical cells, central filaments, radiating filaments, and newly initiated secondary pit connections.

Fig. 17. Longitudinal section of tip showing primary filaments in central core of medulla, radiating filaments forming cortex, and interconnecting secondary filaments.

Fig. 18. Cross-section near tip outlining central core of medulla.

Fig. 19. Cross-section of tip at level of conjunctor cells and earliest secondary pit connections (aniline blue).

Fig. 20. Enlarged view of cells cutting off conjunctor cells (aniline blue).

Fig. 21. Cross-section of tip at level where conjunctor cells have fused onto recipient cells, forming secondary pit connections (aniline blue).

Fig. 22. Enlarged view showing cytoplasmic processes extending from cells at point where conjunctor cells have fused (aniline blue). (Note pit plugs have not yet differentiated within secondary pit connections.)

along the margins of main axes and lateral pinnules. They are terete in cross section and superficially resemble unbranched juvenile thalli. Pinnules cease growing as soon as young cystocarps appear near the tips in female plants, and are usually longer in tetrasporangial plants. Apical cells divide longitudinally with random orientation (Fig. 37), and the primary filaments lose the tendency to converge towards the apex (Fig. 34).

The network of filaments that makes up the medulla is regular and the boundary between medulla and cortex is well defined in pinnules (Figs 34–36). This is partly because growth is confined to a narrow zone just behind the apex. A central core of medullary filaments is lacking, and stellate medullary cells are largely absent (Fig. 34). Nearly every cell produces a 1-celled secondary filament that grows obliquely downwards and connects by means of a terminal conjunctor cell which forms a secondary pit connection with the nearest neighbouring cell below (Figs 35–36). Additional secondary filaments are not as frequent as in the main branches. This pattern of growth results in an open network in the medulla which is surrounded by a cortex of constant thickness composed of regularly branched bead-like filaments.

FEMALE REPRODUCTION: The procarp consists of a supporting cell bearing a horizontally curved 3-celled carpogonial branch and a vegetative cortical filament (Fig. 41). Procarps are initiated at the thallus surface close to the tips of the fertile pinnules (Figs 37–38) and are absent on dichotomously branched main axes. Carpogonial branches are fully formed when the supporting cell lies only 2–3 cell layers below the surface (Fig. 41).

The procarp initial (Fig. 37) is a terminal cell of a leading adaxial cortical filament close to the apex, distinguishable from surrounding outer cortical cells by its larger size and darker staining. The procarp initial divides obliquely by two concavo-convex divisions to produce an intercalary cell (the supporting cell), an adaxial terminal cell (the initial of the carpogonial branch), and an abaxial terminal cell (the initial of a cortical filament).

The 3-celled carpogonial branch is cleaved directly from its initial by two successive anticlinal concavo-convex septa. A carpogonial branch initial first divides into two cells of approximately equal size (Figs 39–40), after which the terminal cell divides unequally at right angles to the first, producing an intercalary cell with a broad base, and a terminal carpogonium with a narrow base (Fig. 41). The first cell of the carpogonial branch is the largest of the three and ovoid to ellipsoid; the second is smaller and ovoid to conical. The carpogonium is usually situated at right angles to the plane passing through the first and second carpogonial branch cells and the sterile cortical filament. It is initially small and sickle-shaped to narrowly oblanceolate and lies directly above the supporting cell. Subsequently, the tip extends producing a club-shaped trichogyne. Cells of the carpogonial branch remain uninucleate until establishment of the trichogyne.

The supporting cell, the first and second cells of the carpogonial branch and the inner cells of the sterile procarpial filament all enlarge, become densely staining and multinucleate, either in the absence (Fig. 42) or presence (Fig. 43) of fertilization. Supporting cells and their carpogonial branches are gradually buried deep inside the cortex as the fertile pinnule continues to grow in

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Figs 23–33. *Gigartina pistillata.* Vegetative morphology of adult thalli (aniline blue) (*Cabioch & Garbary*, 25.vi.1990, NCU).

Fig. 23. Median periclinal section showing core and radiating filaments in a dichotomizing apex.

Fig. 24. Median longitudinal section of apex (perpendicular to plane of thallus compression) showing core and radiating filaments.

Fig. 25. Median periclinal section of cortex and outer medulla in growing region 500 μm behind apex.

Fig. 26. Median periclinal section of central medulla in growing region 500 μm behind apex.

Fig. 27. Median longitudinal section of medulla in growing region 500 μm behind apex.

Fig. 28. Median periclinal section of mature cortex and outer medulla 2 cm behind apex.

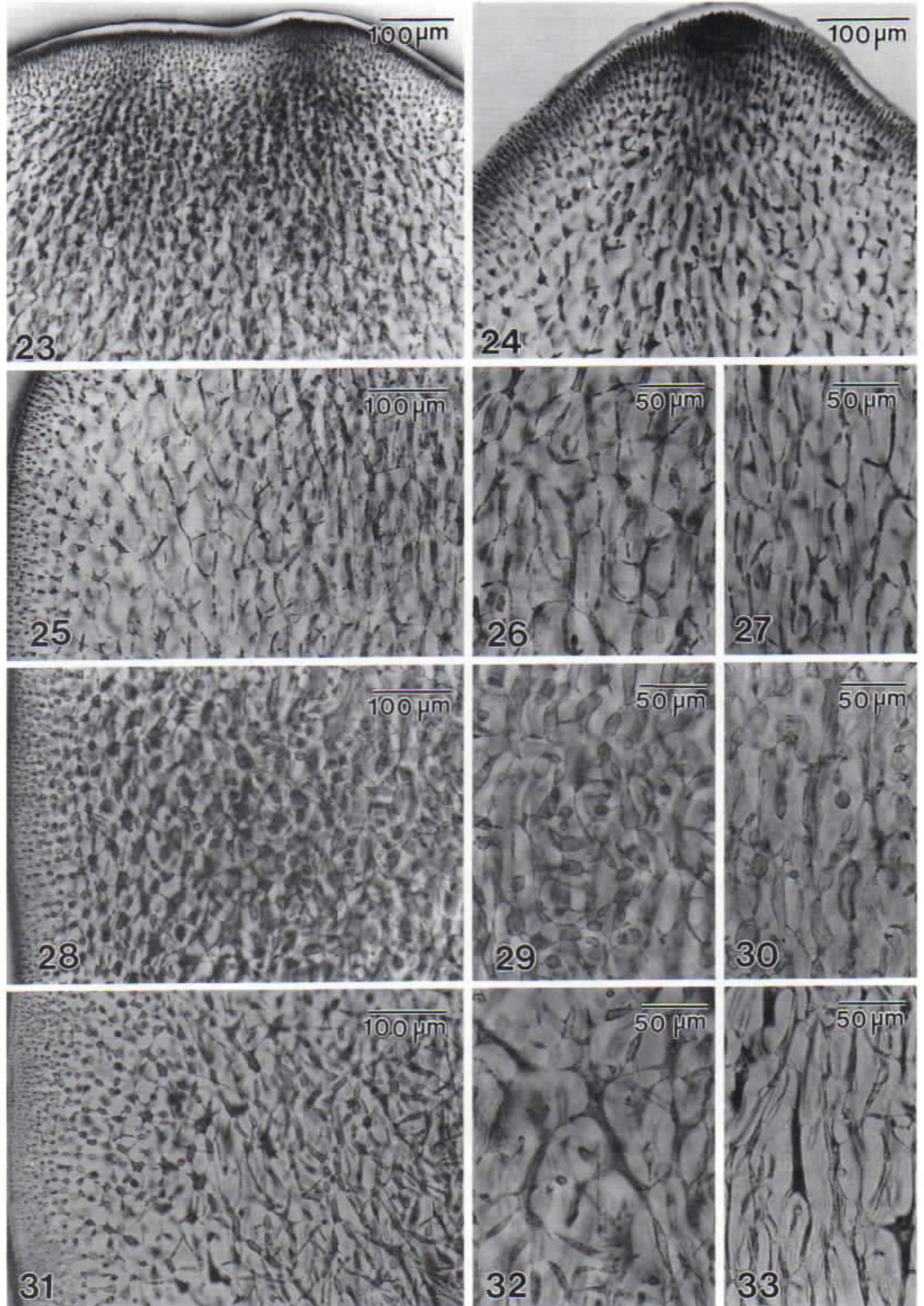
Fig. 29. Median periclinal section of mature central medulla 2 cm behind apex.

Fig. 30. Median longitudinal section of mature central medulla 2 cm behind apex.

Fig. 31. Median periclinal section of cortex and outer medulla in upper part of stipe.

Fig. 32. Median periclinal section of central medulla of upper stipe showing primary (large cells) and secondary filaments (smaller cells).

Fig. 33. Longitudinal section of central medulla in same region as Fig. 32.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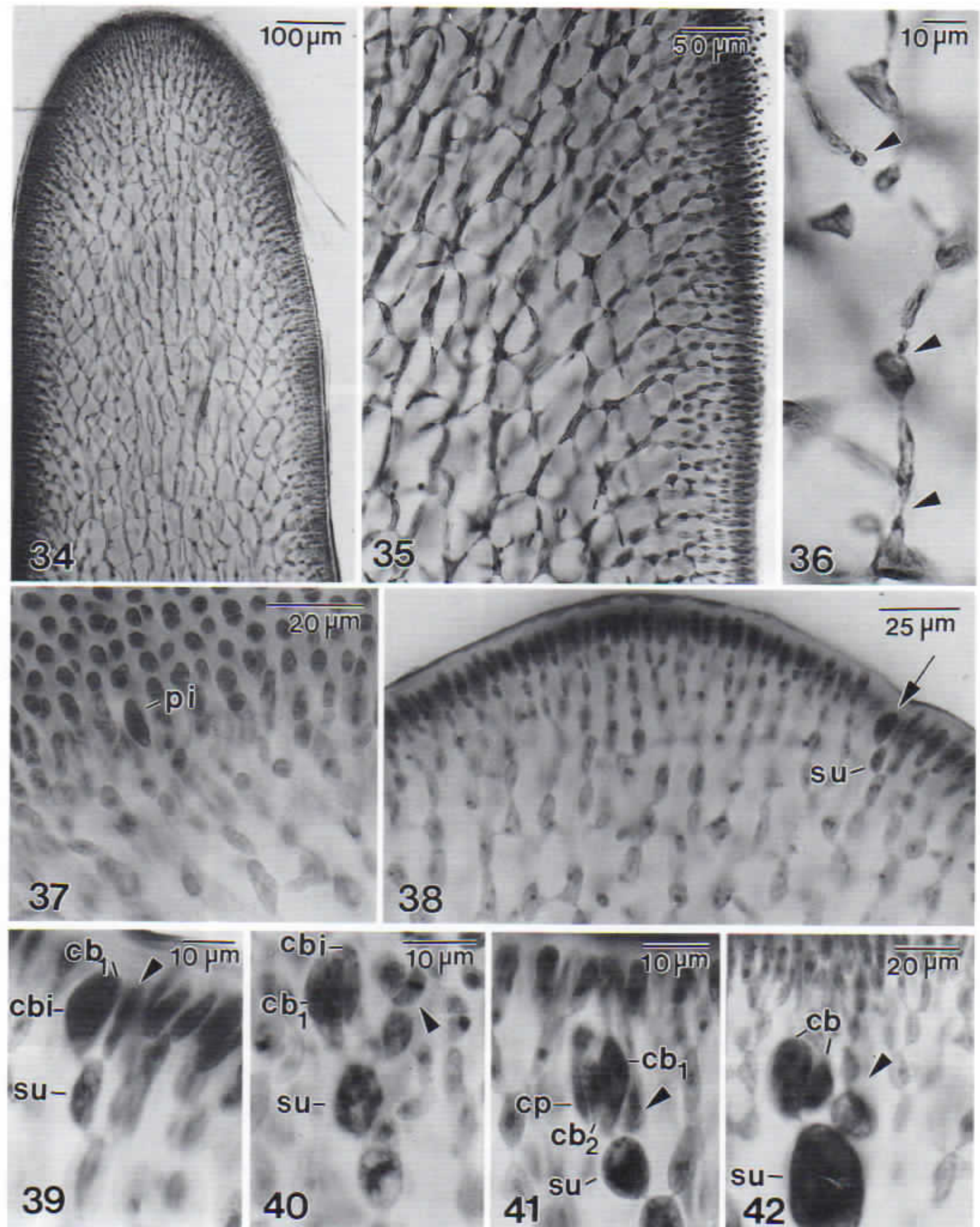

Figs 34–36. *Gigartina pistillata.* Vegetative morphology of pinnules of adult thallus; **Figs 37–42.** Female reproductive system: procarp development (*Cabioch & Garbary*, 25.vi.1990; **Figs 37–40**, *Cabioch*, 14.vii.1991, NCU).

Fig. 34. Median longitudinal section of tip of lateral pinnule.

Fig. 35. Median longitudinal section of cortex and outer medulla 500 μm behind tip.

Fig. 36. Secondary filaments and terminal conjunctor cell (arrowhead, above); fusion of conjunctor cell and recipient cell (arrowhead, middle); fused conjunctor cell (arrowhead, below).

Fig. 37. Procarp initial (pi), a terminal cortical cell near apex. (Note that the longitudinal divisions of apical cells seen above in this grazed section are oriented randomly.)

Fig. 38. Young procarp close to apex of pinnule showing supporting cell (su) bearing two-celled carpogonial branch (arrow).

length and diameter (Fig. 42). Deeply embedded procarps often contain multinucleate carpogonia and are probably non-functional, or the carpogonia abort.

Early post-fertilization stages were rare in our material. Diploidization is achieved when the fertilized carpogonium fuses directly with the supporting cell and apparently transfers its diploid nucleus, becoming enucleate (Fig. 43). Thereafter, the supporting cell functions as an auxiliary cell.

CYSTOCARP DEVELOPMENT: Functional auxiliary cells enlarge irregularly accompanied by the emission of blebs and later of processes from inner and lateral surfaces (Figs 44, 48–49). Such cell extensions lack pit connections and also generally lack nuclei at the time they are produced. Occasionally, detached nuclei are seen in especially large blebs (Fig. 48).

Inner cortical and medullary cells surrounding the auxiliary cell dedifferentiate and cut off initials on all sides that form richly branched filaments surrounding each cell (Fig. 44). Each of these filaments grows by means of an apical cell, intercalary cell divisions being absent. Cortical and medullary cells are evidently induced to form clusters of adventitious filaments in response to a signal radiating from the auxiliary cell. The first clusters are produced from cells next to the auxiliary cell, the next from a shell of cells around the first and so on, until a massive, enveloping secondary tissue surrounds the auxiliary cell. At first, an individual cluster of filaments can be identified with the vegetative cell that produced it (Fig. 44). As the filaments grow towards one another and merge, the individuality of each cluster is gradually lost until a single urn-shaped tissue is produced that envelopes the auxiliary cell (Figs 48–49).

A developing auxiliary cell and its associated tissue commonly aborts. When this happens, cells of the enveloping tissue lose their staining properties and become highly vacuolate. Some cells situated next to the auxiliary cell form filaments that link to it by means of secondary pit connections (Fig. 47). The auxiliary cell ceases growth, and the surrounding cells behave like wound tissue in the repair of the damaged area, converting it back into vegetative tissue.

As the auxiliary cell increases in size, the nuclei align in a central cylinder or column extending longitudinally from the inner to the outer edge of the cell, surrounded by smooth, non-granular cytoplasm (Figs 45, 48). The row of cells corresponding to the original sterile procarpial filament borne on the supporting cell is modified, with each cell enlarging and containing increased numbers of enlarged nuclei (Figs 43–45, 48–49). The tapering row of enlarged cells thus formed defines the apex and future ostiole of the mature cystocarp.

Functional auxiliary cells contain two size categories of nuclei (Figs 45–46): large nuclei, 5–6 μm in diameter, with numerous dot-like chromocentres that stain darkly with haematoxylin, and small nuclei, 2.5 μm in diameter, each with a single dot-like chromocentre. The large nuclei correspond to nuclei present at maturity in a supporting cell bearing a carpogonial branch and carpogonium that had not been fertilized, and they are presumed to be haploid. The small nuclei with the single chromocentre are absent in ordinary supporting cells, and are presumed to be derivatives of the fertilization nucleus deposited into the auxiliary cell after fusion with a fertilized carpogonium. Such nuclei are presumed to be diploid. At first, there appear to be many more haploid than diploid nuclei (Figs 44–45). The diploid nuclei apparently multiply and migrate out of the central column into the surrounding cytoplasm, and ultimately into the processes extending from the base and sides of the auxiliary cell (Fig. 49).

Processes that were initially enucleate acquire diploid nuclei and cut off the initials of gonimoblast filaments. A process and its gonimoblast

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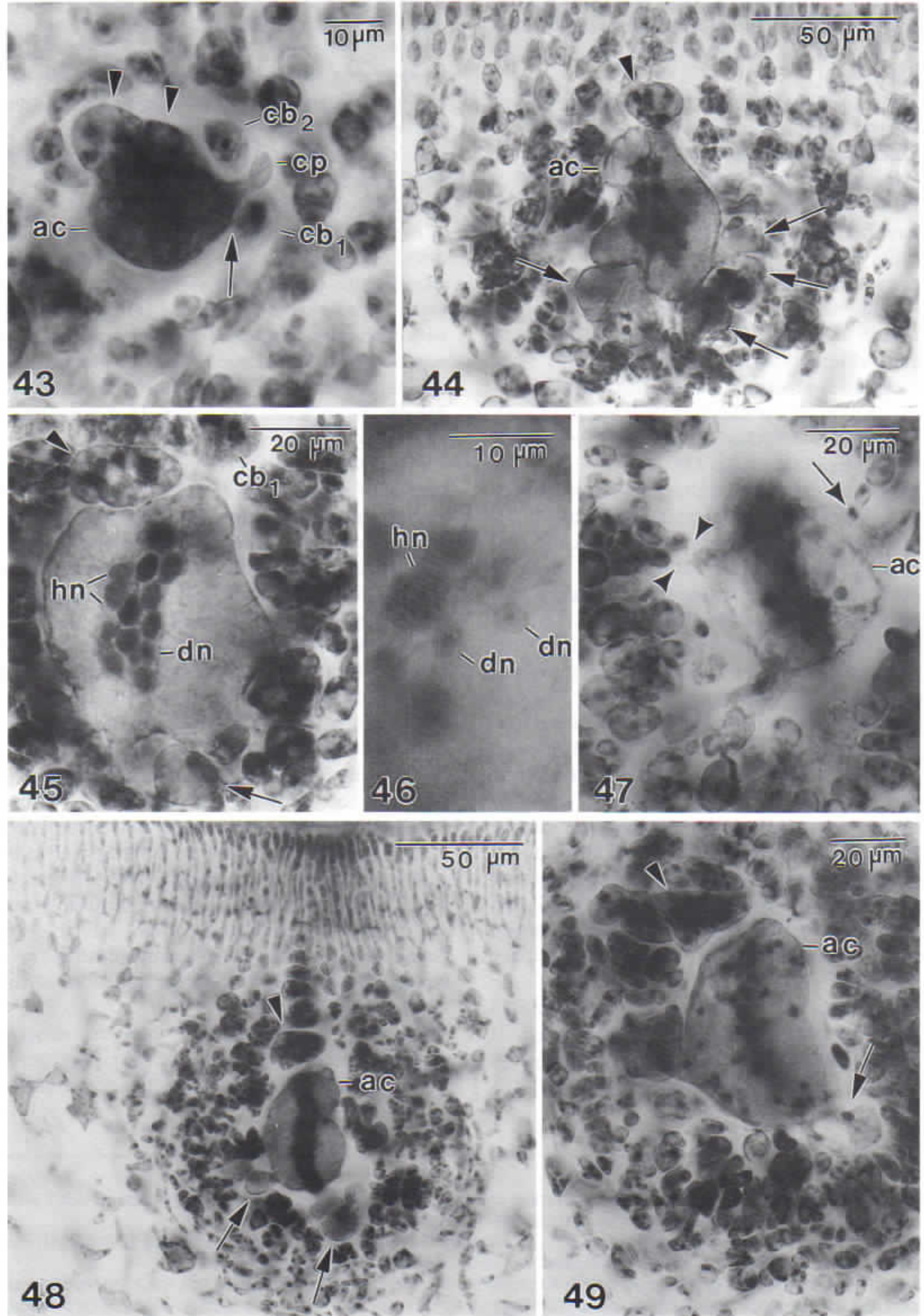
Fig. 39. Procarp of Fig. 38 showing supporting cell (su) bearing first cell of carpogonial branch (cb_1) with carpogonial initial (cbi), and procarpial cortical initial (arrowhead).

Fig. 40. Supporting cell (su) bearing first cell of carpogonial branch (cb_1), carpogonial branch initial (cbi), and procarpial cortical filament with metaphase plate (arrowhead) in subterminal cell.

Fig. 41. Supporting cell (su) bearing 3-celled carpogonial branch consisting of: basal cell (cb_1), intercalary cell (cb_2) and carpogonium (cp) with terminal club-shaped trichogyne, and procarpial cortical filament (arrowhead in Fig. 49) (*Cabioch & Garbary* 25.vi.1990, NCU).

Fig. 42. Non-functional procarp. Enlarged supporting cell (su) bearing degenerated carpogonial branch (cb), and procarpial cortical filament (arrowhead) (*Cabioch & Garbary*, 25.vi.1990, NCU).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filaments often protrude deep inside the enveloping tissue, and the earliest gonimoblast filaments may be almost indistinguishable from the surrounding gametophytic cells (Fig. 50). Gonimoblasts are recognizable at this stage because the gametophytic cells are mostly multinucleate, whereas the gonimoblast cells are uninucleate. The nuclei inside the gonimoblast cells are larger than the original diploid nuclei inside the auxiliary cell, and are a little less than twice the diameter of the haploid nuclei inside cells of the enveloping tissue (3.5–4 μm vs 2–2.5 μm). Several stainable chromocentres can be resolved inside a diploid nucleus of a gonimoblast cell, but there are never as many chromocentres as in the haploid nuclei in the auxiliary cell.

Even very young gonimoblast cells become linked to gametophytic cells of the enveloping tissue, either by cell fusion or by means of secondary pit connections (Fig. 51). Anastomosis can take place in either direction. Conjunctor cells cut off from gametophytic cells can fuse with gonimoblast cells, depositing their nuclei (Figs 51, 54); conversely, gonimoblast cells may fuse directly with a gametophytic cell (Fig. 51), or cut off a conjunctor cell that fuses with one, forming a secondary pit connection (Fig. 54). Occasionally, branched gonimoblast filaments that have not yet become linked to vegetative cells can be clearly seen (Fig. 52); however, gonimoblast and enveloping gametophytic tissues are so thoroughly enmeshed and interlinked most of the time that the two can hardly be distinguished (Figs 53–54). In general, gonimoblast tissues stain more darkly with haematoxylin than enveloping tissues. Some gonimoblast cells, including all potential carposporangia, are uninucleate; however, multinucleate cells may contain only haploid nuclei (like those of the original enveloping tissue), or may contain a mixture of haploid and diploid nuclei as a result of anastomosis. Such a tissue formed by secondary pit connections between gonimoblast and gametophytic cells is called a placenta (Kraft 1978; Hommersand & Fredericq 1990).

Some cystocarps mature early while still relatively small. In such weakly developed cystocarps, terminal gonimoblast cells fuse readily with gametophytic cells at the periphery of the cystocarp and the carposporangia differentiate at an early stage (Fig. 55). The remaining multinucleate cells form a network of elongated cells between the carposporangial chains, like those seen only at late stages in typical cystocarps. Differentiation of the carposporangia is delayed in the larger, more robust cystocarps, and the placenta consists of darkly staining gonimoblast cells and lighter-staining placental cells surrounded by enveloping tissue (Fig. 53). As development proceeds, the enveloping tissue differentiates into an inner translucent ring in which the cell contents have been depleted, and a darkly staining outer ring (Fig. 56). The inner translucent and outer dark rings move steadily outwards as the cystocarp increases in overall size due to the progressive formation and consumption of enveloping tissue. Terminal gonimoblast cells at the periphery of the placenta extend a short distance,

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Figs 43–49. *Gigartina pistillata.* Female reproductive system: early post-fertilization stages (specimens at NCU).

Fig. 43. Bottom view. Diploidization of auxiliary cell. Carpogonium (cp) situated between the first two cells of carpogonial branch (cb_1, cb_2) fused directly to supporting cell (= auxiliary cell, ac). In focus are enlarged cells (arrowheads) of procarpial cortical filament, and pit connection (arrow) between auxiliary cell and cb_1 (the trichogyne is extended towards the thallus surface) (*Cabioch*, 14.vii.1991).

Fig. 44. Multinucleate auxiliary cell (ac) distally connected to enlarged cell (arrowhead) of procarpial cortical filament, and forming enucleate blebs laterally and inwardly (arrows). Branched filaments develop secondarily from surrounding vegetative cells (*Cabioch & Garbary*, 25.vi.1990).

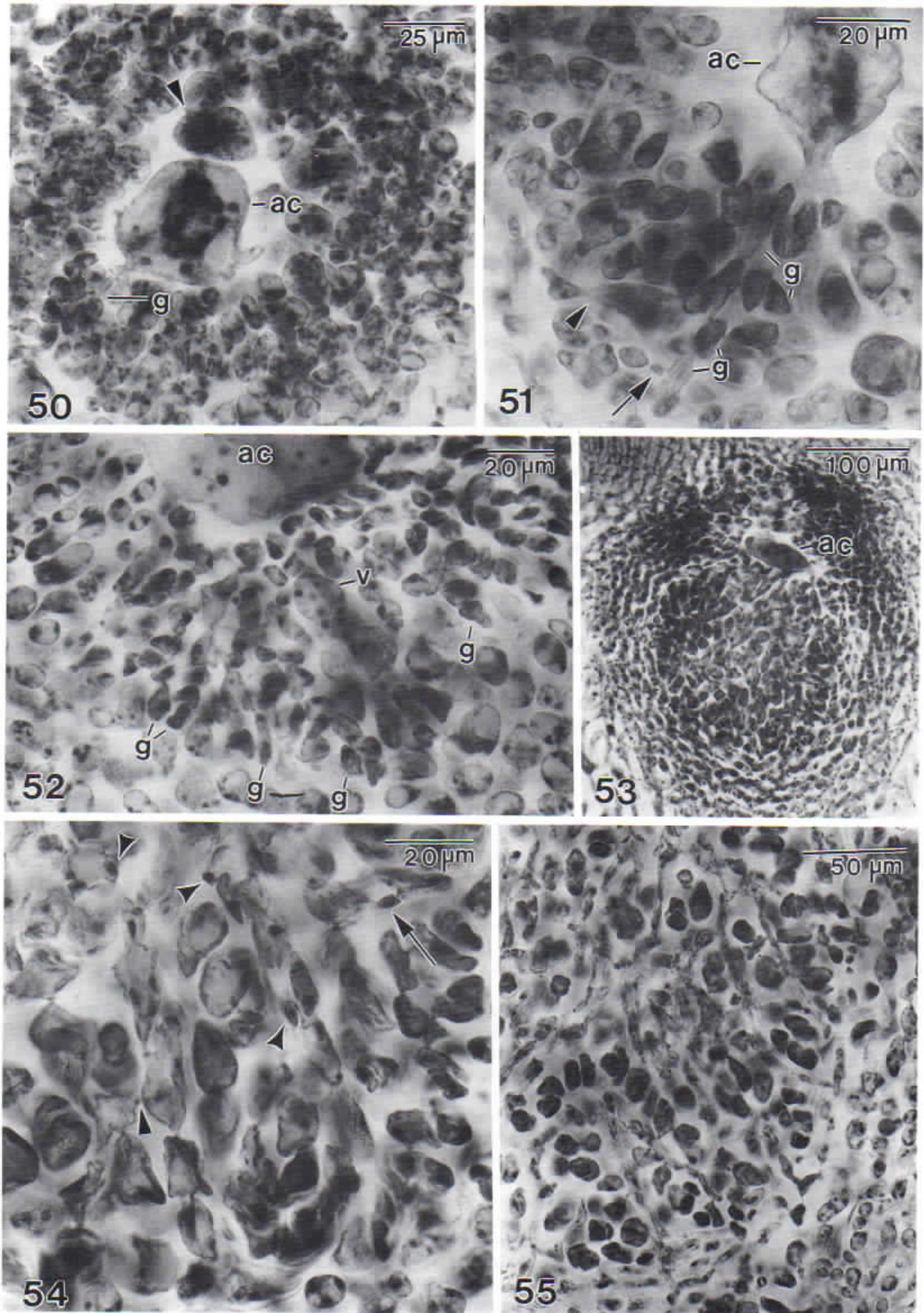
Fig. 45. Auxiliary cell containing mixture of centrally positioned haploid (hn) and diploid (dn) nuclei, with enucleate blebs (arrow), and connected to enlarged cell (arrowhead) of procarpial cortical filament. First cell of carpogonial branch (cb_1) persists (*Cabioch & Garbary*, 25.vi.1990).

Fig. 46. Portion of auxiliary cell showing enlarged, haploid nucleus (hn) with many chromocentres and small, diploid nuclei (dn) with single chromocentre (*Cabioch*, 12.vi.1990).

Fig. 47. Vegetative cells surrounding degenerating auxiliary cell (ac) emitting conjunctor cells (arrow) towards it which form secondary pit connections upon fusion (arrowheads) (*Cabioch & Garbary*, 25.vi.1990).

Fig. 48. Auxiliary cell (ac) containing nuclei organized in central column, with inwardly directed enucleate and nucleated blebs (arrows), surrounded by enveloping tissue and connected to enlarged cells of procarpial cortical filament (arrowhead) (*Cabioch & Garbary*, 25.vi.1990).

Fig. 49. Auxiliary cell (ac) connected to enlarged cell (arrowhead) of procarpial filament. Diploid nuclei have dispersed from central column and one has entered a protrusion (arrow) (*Cabioch & Garbary*, 25.vi.1990).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become tubular, and fuse directly with the gametophytic cells of the surrounding enveloping tissue (Fig. 57).

Everywhere throughout a mature cystocarp, conjunctor cells are cut off from gonimoblast cells (Fig. 58) and fuse to placental cells (Figs 59–60). Placental cells are thus heterokaryotic, and contain a mixture of larger, more darkly staining diploid nuclei (3.5–4 μm) and smaller, less conspicuous haploid nuclei (2–2.5 μm) (Figs 61–64). Like gonimoblast cells, heterokaryotic placental cells may also bud initials (Fig. 61) that form short chains of carposporangia (Fig. 62) or link to other placental cells (Fig. 60). Vegetative cells that behave in this manner usually contain a preponderance of small, haploid nuclei and a few larger, diploid nuclei. Single, diploid nuclei inside carposporangia reach the largest diameters (4–5 μm) (Figs 63–64).

Carposporangial chains may be only 2–3 cells long (Fig. 62), or, more often, are highly branched and form botryoid clusters separated by placental tissue (Fig. 64). Older, more mature cystocarps tend to have masses of carposporangia lining the enveloping tissue at the periphery and grape-like clusters inside separated by placental tissue (Fig. 65). Cells in both the inner, translucent and the outer, darkly staining layers of the enveloping tissue elongate periclinally as the envelope stretches (Figs 65–66). Placental cells connected to the outer envelope also elongate and pit connections between the cells broaden (Figs 66–67). Some of the potential carposporangia cut off cells terminally that extend and are incorporated into the placental network upon fusion (Figs 66–67).

Carposporangia mature more or less simultaneously and are evidently released together through an ostiole which forms in the vicinity of the original sterile procarpial filament borne on the supporting cell (cf Figs 48, 65, and 68). Figure 68 illustrates a cystocarp that has released its carposporangia and is in the process of tissue repair. Release of the carposporangia leaves behind a reticulum of sterile placental cells separated by empty spaces. Most of these cells contain a mixture of haploid and diploid nuclei. The placental cells enlarge and cut off one or several new gonimoblast cells that immediately link up with the formation of secondary pit connections. Such new gonimoblast cells may become multinucleate and do not appear to mature into carposporangia (Figs 69–71). We have not seen any instance of the production of a second crop of functional carposporangia in old cystocarps, even though such cystocarps appear to persist for a long time.

MALE REPRODUCTION: All of the specimens examined bearing male reproductive structures were monoecious. Spermatangial parent cells are transformed surface cortical cells (Figs 72–73) produced at the tips of pinnules bearing young cystocarps. Each spermatangial parent cell cuts off two spermatangia distally by sequential, oblique concavo-convex longitudinal divisions, one on each side (Fig. 73). Spermatangia (Figs 72–73) are subspherical, hyaline, and uninucleate with a centrally located nucleus, and are 3–5 μm in diameter.

TETRASPORANGIAL REPRODUCTION: Tetrasporangia are borne in raised, elliptical to linear lens-shaped sori forming discontinuous patches on all orders of branches except the stipe (Figs 2, 4–5). Individual sori vary in length, width and thickness. Some are relatively narrow, occupying less than a quarter the circumference of a branch (Fig. 74); others are broader, extending over half the circumference of a branch (Fig. 75).

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Figs 50–55. *Gigartina pistillata.* Female reproductive system. Young cystocarps (*Cabioch & Garbary,* 25.vi.1990, NCU).

Fig. 50. Auxiliary cell (ac) connected to enlarged cell (arrowhead) of procarpial cortical filament, with protrusions bearing gonimoblasts (g) penetrating enveloping tissue inwardly.

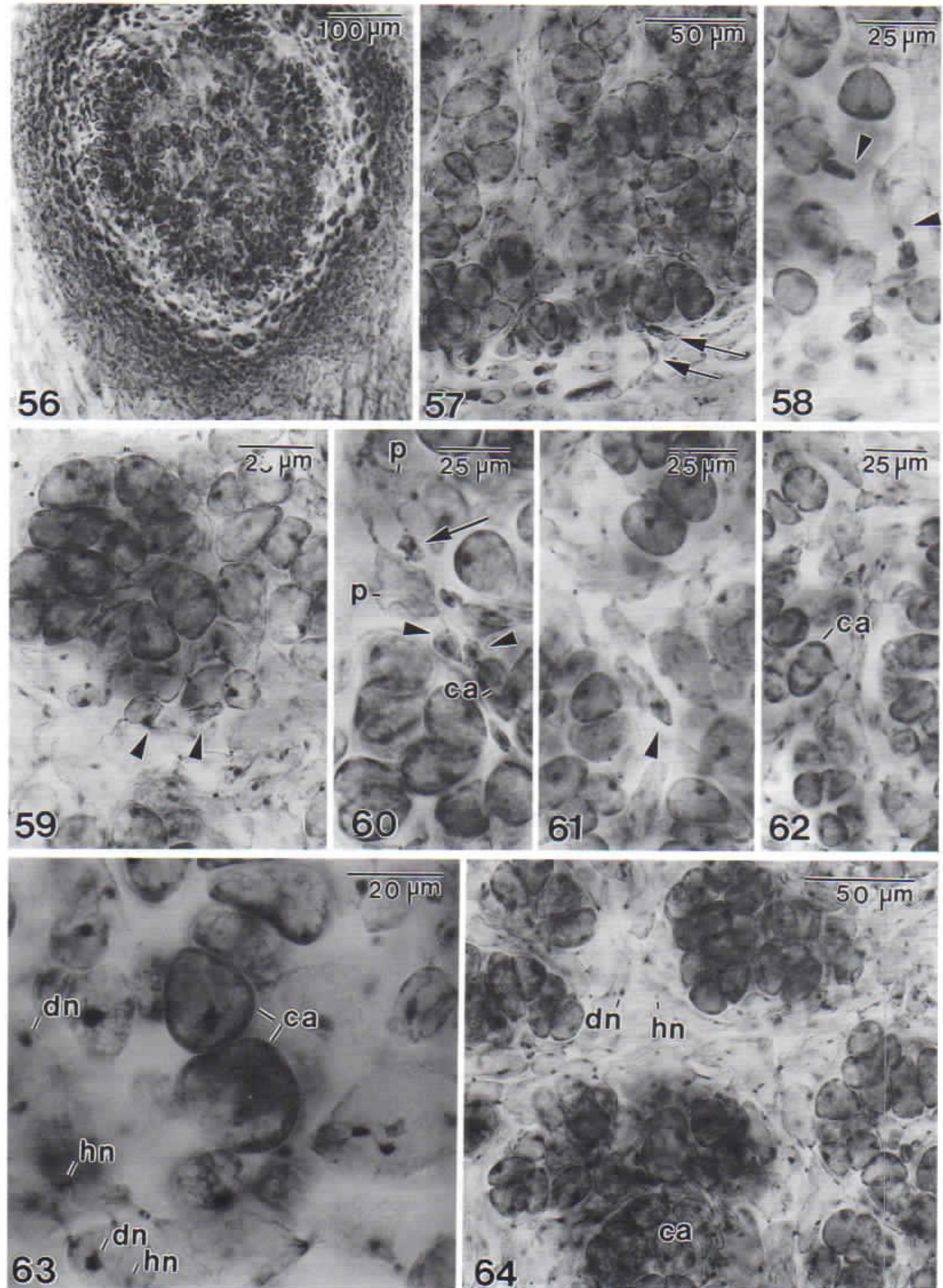
Fig. 51. Auxiliary cell (ac) bearing gonimoblasts (g) showing gonimoblast cell fusing directly to cell of enveloping tissue (arrow), and conjunctor cell cut off from cell of enveloping tissue fusing with gonimoblast cell (arrowhead).

Fig. 52. Auxiliary cell (ac) bearing gonimoblast filaments (g) not yet fused to vegetative cells (v).

Fig. 53. Auxiliary cell (ac) bearing gonimoblasts (dark-staining areas) penetrating enveloping tissue (light-staining areas) forming central placenta surrounded by outer ring of enveloping tissue.

Fig. 54. Development of placenta in central area of Fig. 53. Conjunctor cell (arrow) cut off from cell of enveloping tissue fusing with gonimoblast cell, and conjunctor cells cut off from gonimoblast cells (curved-sided arrowheads) interlinking by secondary pit connections (straight-sided arrowhead).

Fig. 55. Margin of weakly developed cystocarp showing narrow placental cells bearing carposporangial initials, and fusing terminally to cells of enveloping tissue.



Figs 56–64. *Gigartina pistillata.* Female reproductive system: maturing cystocarps (*Cabioch & Garbary*, 25.vi.1990, NCU).

Fig. 56. Placenta bearing young carposporangia surrounded by massive enveloping tissue. Cells of lighter-staining inner ring have depleted contents.

Fig. 57. Terminal placental cells producing tubular cells (arrows) fusing to cells of enveloping tissue.

A sorus is initiated at the boundary between cortex and medulla and grows in thickness as a result of the addition of new tetrasporangial tissues at the boundary (Figs 76–78). Tetrasporocytes originate in two ways: firstly as transformed intercalary cortical cells in a primary cortical filament, and secondly as transformed cells in secondary filaments derived laterally from primary or other secondary filaments. Potential tetrasporocytes are roundish, stain more darkly than vegetative cells and contain a single, conspicuous, spherical nucleus. Vegetative cells at the inner boundary of the cortex are elongate to irregular in shape, stain lightly, are vacuolate and contain several small nuclei.

The outer cortex remains constant in thickness at 7–8 cell layers as the tetrasporangial sorus increases in size. Inner, uninucleate primary cortical cells cut off initials laterally (Fig. 76) which form filaments 2–4 cells long (Figs 77–78). As the terminal cell of such a filament approaches or makes contact with a neighbouring cell, it cuts off either small, lenticular conjunctor cells, or elongate tubular cells that fuse with the recipient cell, depositing its nucleus (Figs 79–80). Any uninucleate cell in a secondary filament may branch laterally, producing additional secondary filaments until all available space is filled with a network of short chains of tetrasporocytes linked to multinucleate cells by secondary pit connections (Figs 78–80). Only uninucleate cells become tetrasporocytes. Multinucleate vegetative cells and any cell that becomes multinucleate after receiving a nucleus from a conjunctor cell does not become a tetrasporocyte, but will function as a vegetative cell. Up to six conjunctor cells cut off by potential tetrasporocytes may fuse with a single recipient cell.

Tetrasporocytes cleave successively to produce four cruciately arranged tetraspores (Figs 81–83). Tetrasporangia average 30–50 μm × 20–30 μm and remain pit-connected in chains (Fig. 82). Vegetative cells above and between tetrasporangia or connected to them become slender and elongated as the sorus matures (Fig. 83).

The sporangia in each are released simultaneously, leaving behind an eroded surface (Figs 5, 85). The excision takes place at the boundary between the innermost tetrasporangia and a cluster of small, darkly staining cells formed within secondary filaments in the medulla below (Fig. 84). An abscission layer is not produced prior to release. Instead, a gelatinous material condenses at the exposed surface (Fig. 86), suggesting that sorus release involves excretion of large amounts of gelatinous substance at the site of excision. The darkly staining cells beneath the sorus may be the source of this material. A darkly staining cuticle forms soon afterwards, covering the exposed surface with a protective layer (Figs 87–88).

New filaments arise adventitiously and grow anticlinally, producing a somewhat irregular secondary cortex (Fig. 88). In this way, the entire surface that was formerly the inner boundary of a tetrasporangial sorus is repaired. New sori form adjacent to the area occupied by the released sorus (Fig. 85). When they, in turn, are released, the process is repeated until virtually the entire surface area of the tetrasporangial thallus except for the stipe is consumed. Some tetrasporangial plants appear to have released tetraspores over more than one season.

DISCUSSION

Vegetative development

Kylin (1923, 1956) characterized vegetative development in the Gigartinaceae as an example of 'Springbrunnentypus' (fountain-type) in which the medulla and inner cortex form a loose, filamentous network and the outer cortex consists of compact small-celled anticlinally arranged fil-

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Fig. 58. Potential carposporangia cutting off 2-celled chains including terminal conjunctor cell (arrowhead).

Fig. 59. Carposporangial cluster with potential carposporangia cutting off conjunctor cells (arrowheads).

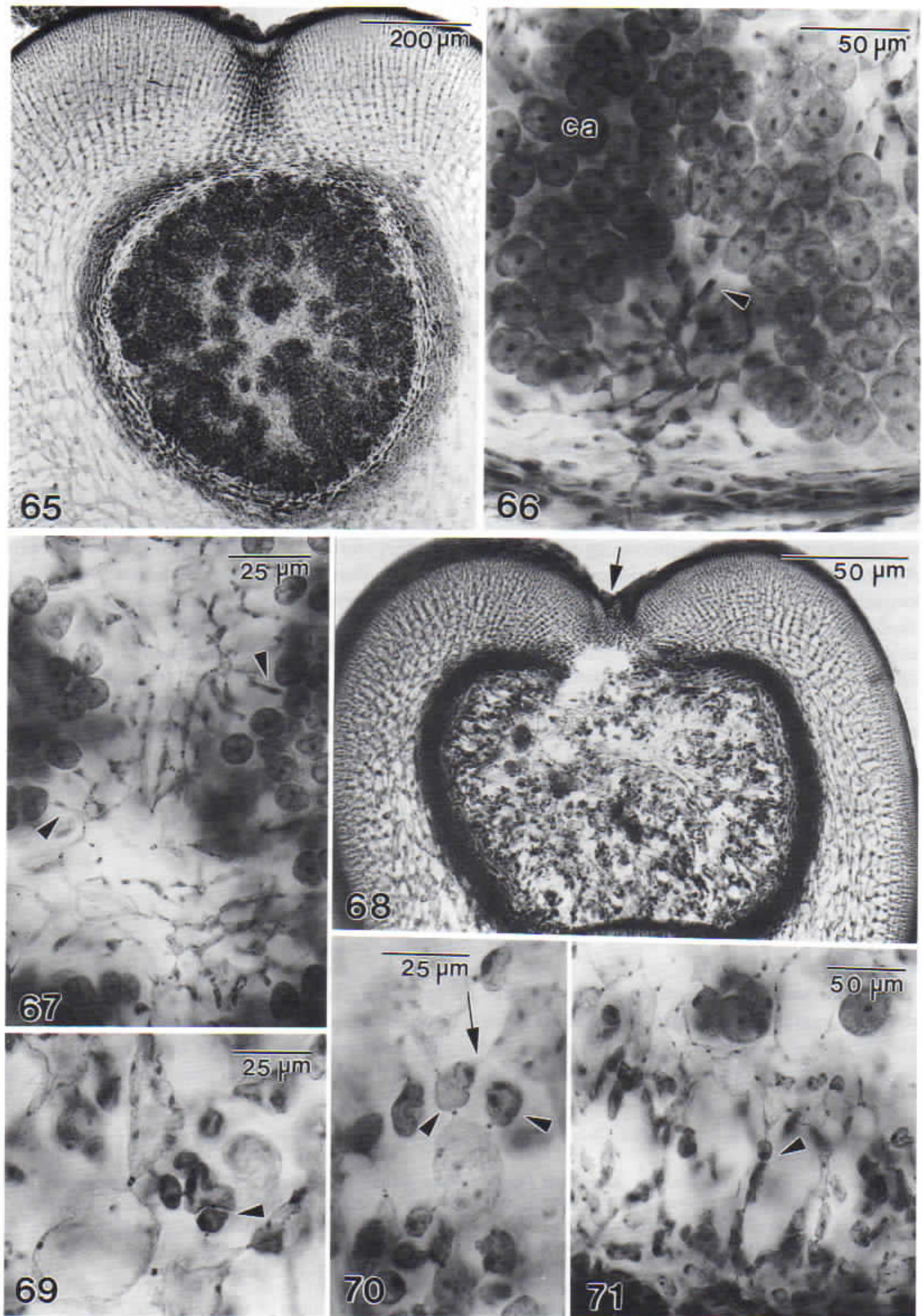
Fig. 60. Cell containing diploid nucleus linking between potential carposporangium (ca) and placental cell (p) (arrowheads) and between two placental cells (arrow).

Fig. 61. Placental cell cutting off carposporangial initial (arrowhead).

Fig. 62. Two-celled carposporangial chain (ca) borne on placental cell.

Fig. 63. Carposporangia associated with placental cells containing mixture of small, haploid (hn) and larger, diploid (dn) nuclei.

Fig. 64. Carposporangial clusters separated by placental cells containing mixture of small, haploid (hn) and larger, diploid (dn) nuclei.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aments. Several families of fleshy red algae, most notably the Halymeniaceae, contain genera in which the medulla and inner cortex consist of a loose network of interconnected cells (e.g., Kylin 1956); however, it is rare to see a network formed so close to the apical meristem as in *Gigartina pistillata*.

The juvenile thallus of *Gigartina pistillata* exemplifies a form of 'fountain-type' growth that was first described by Oltmanns (1904, pp. 544–546; 1922, pp. 259–261) in *Furcellaria lumbricalis* (Hudson) Lamouroux (as *F. fastigiata*). Growth is monopodial and pseudodichotomous in *Furcellaria*. Two concavo-convex divisions initiate a branched filament. The apical cell produced by the first division is directed adaxially towards the centre of the apex, while the second apical cell is cut off abaxially and initiates a radiating filament. In *Furcellaria*, the core of the medulla derived from the central cluster of apical initials consists of narrow, elongated filaments surrounded by an inner cortex of inflated, radiating filaments, and secondary pit connections are absent. In *G. pistillata*, on the other hand, the central core is recognizable only at the tip, just behind the apical meristem. Secondary pit connections are formed close to the apex, a primary network is generated, and longitudinal and radial filaments soon become indistinct.

Vegetative growth of the Gigartinaceae has received scant attention in the literature. Darbishire (1902) and Kylin (1923) illustrated the primary network in the growing apex in *Chondrus*, and longitudinal sections of germlings arising from crusts have been described in *Chondrus* (Chen & McLachlan 1972; Prince & Kingsbury 1973); however, the behaviour of conjunctor cells in network formation is described and illustrated here for the first time in a member of the Gigartinaceae. Most drawings and photographs do not show all of the pit connections between neighbouring cells; however, critical observation reveals that every cell is usually connected to each of its neighbouring cells by a single primary or secondary pit connection. Formation of secondary pit connections, cell enlargement, and the accumulation of matrix material in the intercellular spaces combine to produce the regular primary network characteristic of the Gigartinaceae.

Secondary medullary filaments up to three cells long link by secondary pit connections to preexisting cells a short distance behind the apex. These are uninflated in the pinnules of *G. pistillata*, and maintain a net-like appearance much like that seen in the principal branches of thalli of many other species of Gigartinaceae. The inflated condition of mature primary and secondary filaments is less common, and its extreme form may be peculiar to *G. pistillata*.

Primary branching is dichotomous in *G. pistillata*, and involves the separation of apical initials and their derivatives into two masses at the time of branching, with the pinnules arising adventitiously from the cortex. Not all Gigartinaceae develop in this manner. Many have extended growing regions, sometimes covering nearly the entire thallus (Norris & Kim 1972). Whether the meristem is apical, marginal, or diffuse, the filaments grow in the same way. As new filaments are interpolated during branching at the surface, inner cortical cells are steadily converted into medullary cells. Only the medulla increases in breadth, while the cortex remains relatively constant in thickness.

Most species of the Gigartinaceae form discoid or crustose holdfasts of one sort or another that give rise to erect juvenile thalli, much as in *G. pistillata*. Zollner (1977) investigated the juvenile thallus in a range of species belonging to the Gigartinaceae. In all of them, the juvenile upright was unbranched and anatomically distinct from

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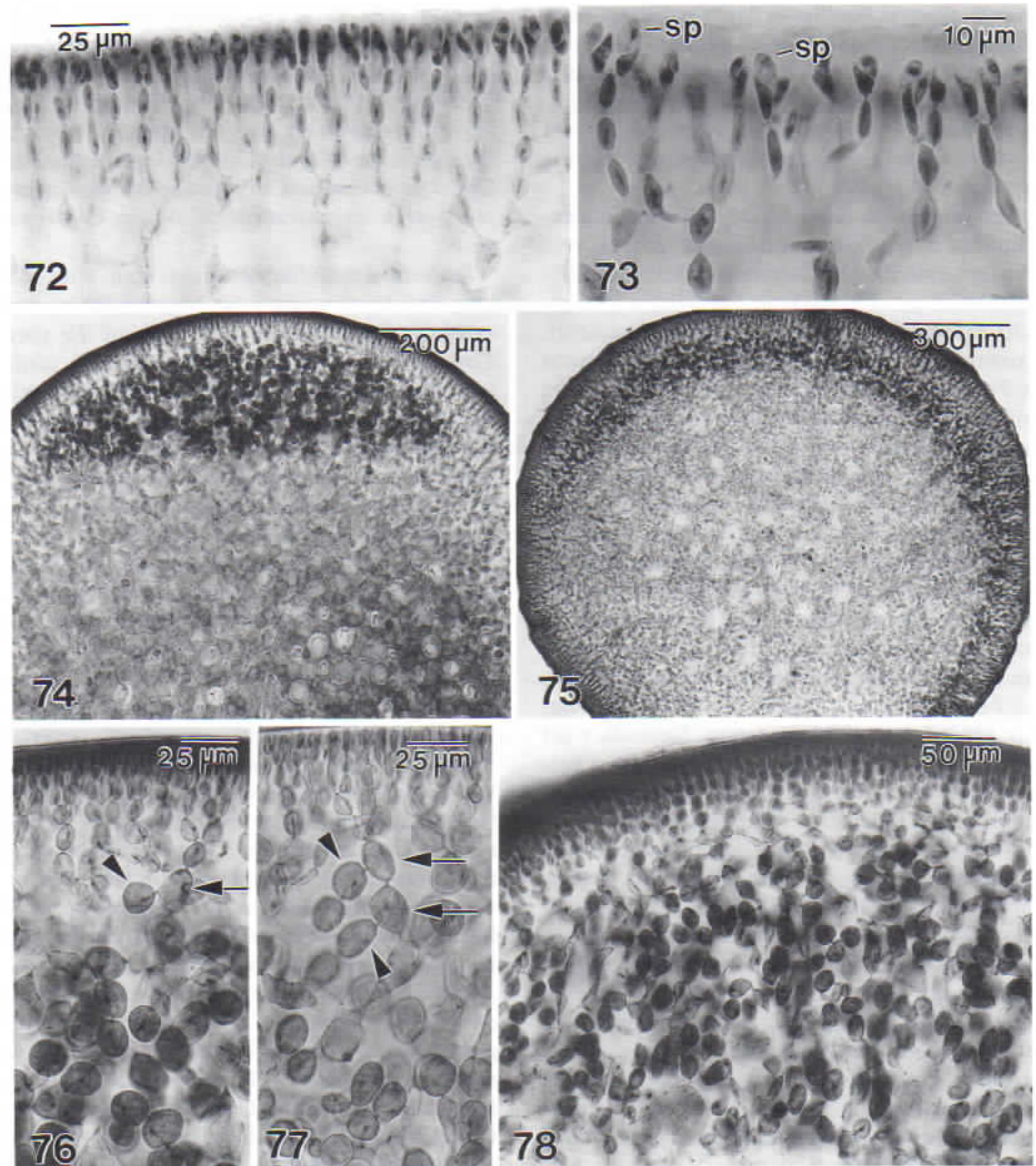
Figs 65–71. *Gigartina pistillata*. Female reproductive system: mature cystocarps (*Cabioch & Garbary*, 25.vi.1990, NCU).

Fig. 65. Mature cystocarp with massive enveloping tissue surrounding placenta with clusters of carposporangia.
Fig. 66. Carposporangia (ca) bearing elongated cells (arrowhead), linked to placental network of narrow filaments connected to outer envelope.

Fig. 67. Network of placental cells with enlarged pit connections and carposporangia bearing elongated cells (arrowheads) linked to placental network.

Fig. 68. Empty cystocarp with sunken ostiole (arrow) and placenta undergoing tissue repair surrounded by envelope.

Figs 69–71. Close-up of Fig. 68 showing second crop of carposporangial analogues (arrow) originating from placental cells and linked by secondary pit connections to neighbouring cells (arrowheads).



Figs 72–78. *Gigartina pistillata.* Male and tetrasporangial reproductive systems (specimens at NCU).

Figs 72–73. Male reproductive system. Spermatangia (sp) produced from terminal cortical cells (*Cabioch & Garbary*, 25.vi.1990).

Figs 74–78. Tetrasporangial reproductive system (*Cabioch*, 19.iii.91).

Fig. 74. Young, narrow sorus of tetrasporocytes.

Fig. 75. Young, broad sorus of tetrasporocytes.

Fig. 76. Secondary tetrasporocyte (arrowhead) originating laterally from primary tetrasporocyte (arrow).

Fig. 77. Secondary 2-celled tetrasporocyte filaments (arrowheads) originating from primary tetrasporocytes (arrows).

Fig. 78. Young sorus showing mostly secondary tetrasporocyte filaments.

the adult thallus. Transformation of the juvenile into the adult thallus involves subtle changes in meristem activity that alter the growth process and anatomical features of the differentiated tissues. In general, tissues present in a juvenile upright are more compact, reflecting the cartilaginous consistency of the mature stipe as compared with the more fleshy consistency of the adult thallus.

Our observations, along with those in the published literature, suggest that the central features of vegetative development described here are highly conserved in members of the Gigartinaceae, perhaps more so than many reproductive characters.

Reproductive development

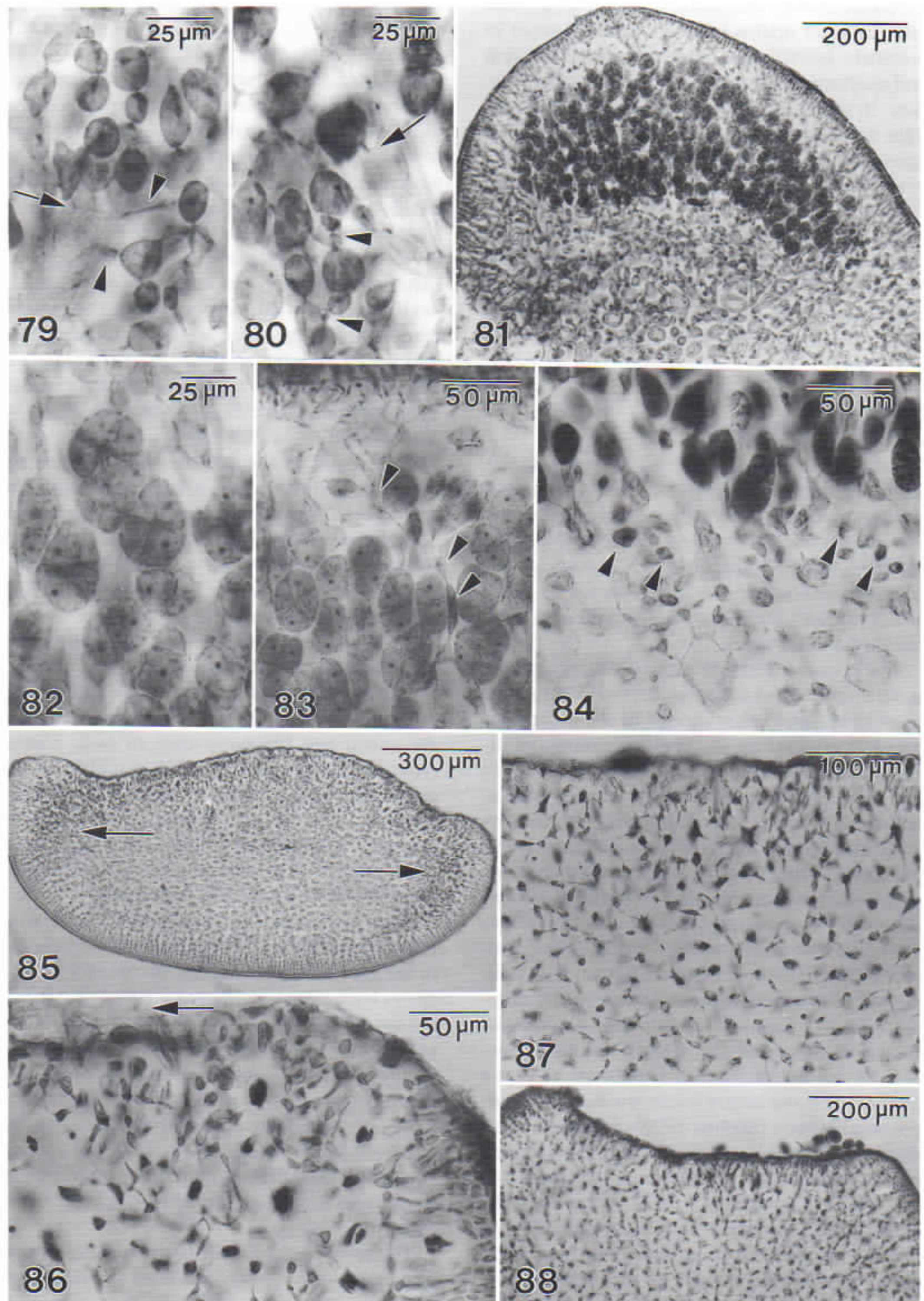
Spermatangia occur at the tips of young, fertile female pinnules in field-collected specimens, similar to those observed by Guiry (personal communication) in cultured plants of *Gigartina pistillata* from Santec, Brittany. Species of the Gigartinaceae are commonly reported as being dioecious, and the monoecious condition of *G. pistillata* is thus distinctive.

The procarp initial is clearly distinguishable as an apical cell of a leading cortical filament in *G. pistillata* and other species in the Gigartinaceae investigated by us. The two successive divisions of the procarp initial are concavo-convex and resemble the septation process that leads to branching in a vegetative filament, with the terminal cell producing the carpogonial branch and the lateral initial forming a sterile filament. Homology of the procarp initial with the apical cell of a vegetative cortical filament may well prove to be a special feature of the Gigartinaceae complex (Gigartinaceae, Chondriellaceae, Petrocelidaceae, Phyllophoraceae). The procarp originates from a terminal surface cell in *Champia* and *Lomentaria* of the Rhodymeniales; however, in this case the terminal cell forms a sterile filament and the lateral initial produces the carpogonial branch (Bliding 1928). In several families currently placed in the Gigartinales, the supporting cell appears to be an intercalary cell that cuts off the carpogonial branch initial laterally, or the female reproductive apparatus is borne on a secondary filament that is distinct from ordinary vegetative filaments (see Kylin 1956).

The only records of fertilization in the Gigartinaceae and the Petrocelidaceae come from experimental crosses in species of *Chondrus, Mastocarpus* and *Gigartina* (e.g., Chen & McLachlan 1972; West & Guiry 1982; Guiry & West 1983; Guiry & Cunningham 1984; Guiry *et al.* 1987; Brodie *et al.* 1991). Spermatangia have been seen adhering to trichogynes in *Mastocarpus stellatus* (Stackhouse) Guiry of the Petrocelidaceae (Guiry & West 1983, as *Gigartina stellata*). Our observation of an enucleate carpogonium fused to an auxiliary cell suggests that the diploid nucleus is transferred directly into the auxiliary cell before dividing further. Haploid and diploid nuclei evidently occupy the same volume of cytoplasm in auxiliary cells of all members of the Gigartinaceae complex; however, we have not seen haploid nuclei as large as the ones encountered in the auxiliary cell of *Gigartina pistillata* in any other member of the Gigartinaceae at which we have looked so far. Modified haploid nuclei are seldom found in the auxiliary cell itself in red algae but instead occur in adjoining cells (Dumontiaceae, Kallymeniaceae, Peyssonneliaceae, Solieriaceae, and Plocamiaceae), or in 'sterile groups' or 'nutritive tufts' (Ceramiales, Bonnemaisoniales) or special secondary 'nutritive tissues' (Cystocloniaceae, Rhodymeniaceae, Champiaceae, Lomentariaceae and other families) (Hommersand & Fredericq 1990). The very existence of these alternative strategies implies that separation of diploid and haploid nuclei is the norm, and the occurrence of both nuclear types in mixed array, as in *G. pistillata,* is unusual. Apart from members of the Gigartinaceae complex, haploid and diploid nuclei may be present together in auxiliary cells of the Acrotylaceae, Dicranemaceae and Mychodeaceae (Kraft 1978 and included references).

As we have seen, the small, diploid nuclei multiply in number in *G. pistillata* and migrate out of the central area of the auxiliary cell into processes from which uninucleate gonimoblast cells are cut off. There are occasional illustrations of species of the Gigartinaceae and Petrocelidaceae in the literature showing processes emanating from an enlarged auxiliary cell surrounded by enveloping tissue or modified gametophytic tissue (e.g., Mikami 1965; Kim 1976). In our experience, these figures illustrate a pre-gonimoblast stage before or just after dispersal of the diploid nuclei.

The absence of a special tissue surrounding the gonimoblasts has long been used as a diagnostic character separating *Chondrus* from *Gigartina.* Kim (1976, p. 62) discussed the history of terms applied to the tissue that surrounds the goni-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moblasts in *Gigartina* and proposed that it be called simply 'enveloping tissue'; we have adopted this term here. Kim specifically rejected the terms 'Nährgewebe' (Kylin 1928, p. 48) or 'nutritive tissue' (Fritsch 1945, p. 672) because '. . . nothing is known of its physiological importance'. Physiological data are still lacking, although the circumstantial morphological evidence presented here clearly suggests that the enveloping tissue has a nutritive function. Fusions occur between gonimoblast cells and cells of the enveloping tissue, nuclei are exchanged, and cytoplasm, assessed with regard to its stainability, builds up and disappears as the cystocarp develops and the carposporangia mature.

Kim (1976, Table III, p. 71) used the size and compactness of the enveloping tissue as one of the key characters for separating species of *Gigartina* (in which genus he included the genera *Iridaea* and *Rhodoglossum*). It should be noted, however, that enveloping tissue is not only produced but also consumed in the course of gonimoblast development, and the thickness of the envelope varies depending on the balance achieved between these two processes at any one time.

The production and utilization of the enveloping tissue is a continuous process in *G. pistillata*, involving early linkage between diploid and haploid tissues and progressive modification of both, generating a large cystocarp with many carposporangia. There appears to be just one crop of carpospores which is apparently released at one time. Earlier, Hommersand & Fredericq (1990, table 13-1, p. 342) proposed that evolutionary trends in the nutrition of the carposporophyte progressed from a condition in which the nutritive tissue is preformed with various modifications of cytoplasm, nuclei and pit connections taking place prior to fertilization, to one in which development involves discrete steps in which nutritive tissue is generated and utilized according to some schedule after fertilization. The cystocarp of *G. pistillata* has developed further in that production and consumption of nutritive tissues involves discrete events at some stages, but is otherwise a continuous process involving the formation of an elaborate placenta consisting of gametophytic cells, heterokaryotic placental cells, gonimoblasts and carposporangia. Nothing we have seen in other members of the Gigartinaceae matches the level of complexity encountered in this species.

As was true of the gonimoblasts and carposporangial chains, development of the tetrasporangial sorus and tetrasporangial chains is progressive, beginning at the boundary between cortex and medulla and continuing until the tetraspores mature and are ready for release. A small area of an immature cystocarp or tetrasporangial sorus would be difficult to distinguish under the microscope. Carposporangial and tetrasporangial filaments are each only a few cells long, and are linked by secondary pit connections to vegetative cells. Both carposporangia and tetrasporangia cut off terminal conjunctor cells which may elongate and form additional linkages. Even the vegetative cells elongate and collapse, forming a network of filaments linking the sporangial masses, either with the sterile envelope (cystocarpic plants), or the outer cortex (tetrasporangial plants). The parallels are so striking that one is tempted to suggest that their development is regulated by an identical mechanism. Apparently, in the Gigartinaceae, certain advanced morphological features which evolved in one phase

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Figs 79–88. *Gigartina pistillata.* Tetrasporangial development (*Cabioch,* 19.iii.91, NCU).

Figs 79–80. Terminal cells of tetrasporocyte filaments bearing elongated cells (arrowheads) linked to neighbouring cells leaving behind secondary pit connections (arrows).

Fig. 81. Mature tetrasporangial sorus.

Fig. 82. Chains of tetrasporangia linked by pit connections.

Fig. 83. Tetrasporangia connected to elongated vegetative cells (arrowheads).

Fig. 84. Boundary between innermost tetrasporangial sorus and outer medulla showing small, darkly staining roundish cells (arrowheads) (aniline blue).

Fig. 85. Cross-section showing area of excised tetrasporangial sorus and new sori (arrows) developing laterally (aniline blue).

Fig. 86. Site of excised sorus showing residual gelatinous material (arrow) and incipient tissue repair (aniline blue).

Fig. 87. Newly formed cuticle and tissue repair at site of extruded sorus (aniline blue).

Fig. 88. Cuticle and repaired outer layer resembling a secondary cortex (aniline blue).

of the life history (the carposporophyte) have been transferred laterally to another (the tetrasporophyte).

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