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Mixed-phase reproduction of *Bostrychia* (Ceramiales, Rhodophyta) in culture. I. *B. tenella* (LAMOUROUX) J. Agardh

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Isolates of *Bostrychia tenella* from Puerto Rico and the Philippines were placed into culture and their life histories completed. Tetraspores isolated from a Puerto Rico tetrasporophyte developed into bisexual and unisexual gametophytes that produced carposporophytes only in the presence of spermatia. Carpospores developed into gametophytes (no tetrasporophytes). Tetraspores isolated from a Philippine tetrasporophyte developed into bisexual and unisexual gametophytes, and mixed-phase plants with non-functional tetrasporangia developing on procarpial stichidia of gametophytes. Carpogonial branches on bisexual and unisexual gametophytes, and mixed-phase plants were 2–, 3– or 4–celled. Viable carposporophytes developed on all procarp-bearing plants when spermatia were present. Carpospores isolated from gametophytes and mixed-phase plants did not develop into tetrasporophytes but into bisexual and unisexual gametophytes and mixed-phase plants again.

Key Index Words: Bostrychia—*Ceramiales—mixed-phase reproduction—Philippines—Puerto Rico—Rhodophyta.*

The 18 currently recognized *Bostrychia* species are distributed widely in a range of habitats: cold and warm temperate estuarine marshes and open rocky coast, tropical mangroves and open rocky coast, and even tropical freshwater streams, caves and ponds. In estuarine and marine habitats, plants are subjected to wide ranges of salinity and temperature because of variable seasonal and diurnal periods of emersion and immersion.

The geographic distribution of *Bostrychia* species was treated extensively by POST (1936). *Bostrychia tenella* (LAMOUROUX.) J. AGARDH was first described on the basis of specimens from St. Croix, Virgin Islands (see TAYLOR, 1960) and has been collected in many other localities, as is indicated by the brief listing of recent references below: Australia (KING 1981), Brazil (CORDEIRO-MARINO 1978, OLIVEIRA-FILHO 1977), China (TSENG 1983), Colombia (SCHNETTER and BULA-MEYER 1982), Galapagos (TAYLOR 1945), Hong Kong (TSENG 1943), Japan (TANAKA and CHIHARA 1984b), Micronesia (TSUDA and WRAY 1977), Philippines (SILVA *et al.* 1987), Solomon Islands (WOMERSLEY and BAILEY 1970), Tanzania (JAASUND 1976), Viet-nam (PHAM HOANG 1969), and West Africa (LAWSON and JOHN 1982). KING *et al.* (1988) incorporate *B. binderi* HARVEY and *B. flagellifera* POST into *B. tenella* on the basis of continuous variation in five vegetative morphological characters.

Despite its wide distribution, there are no records of the reproductive phenology or investigations on the life history. Tetrasporangia and cystocarps are recorded occasionally (e.g., BØRGESEN 1918, CHAPMAN 1963, TAYLOR 1960) and spermatangia have been reported once (FALKENBERG 1901), but no observations are available on the procarps.

The relative ease with which *Bostrychia tenella* and other species of the genus may

be grown in defined laboratory conditions affords an excellent opportunity to investigate many aspects of their reproduction.

In this report, we attempt to describe and explain the interesting reproductive patterns of two *Bostrychia tenella* isolates from two geographic areas. These patterns involve gametophyte recycling in both isolates combined with mixed-phase reproduction in one. "Mixed-phase" is a term used by VAN DER MEER and TODD (1977) for red algae which form tetrasporangia and gametangia on the same plant.

Materials and Methods

The Puerto Rico plants (Culture No. 2756) were collected from *Rhizophora mangle* L. roots in a shaded habitat, in Parguera, Puerto Rico, on November 2nd, 1986. This sample was shipped in four days by air mail in a small plastic bag containing paper towel moistened in seawater. This proved to be an inexpensive and successful way to send living specimens for culture work. The Philippine plants (Culture No. 2722) were collected from a tidepool in limestone rock, in Dequey Island, Batanes Province, on May 10th, 1986. The plants were associated with various turf algae and were subjected to full sunlight and wide temperature variation (15–40°C). The samples were stored in wide-mounted 250 m*l* polyethylene bottles in a cool chest for up to three weeks. Water was changed daily and the biomass volume was no greater than 5% of the seawater volume (100 m*l*). The chest and bottles were kept open to air and in subdued light when not in transit. On arrival, all plants were placed in deep storage dishes (Pyrex 3250) with 200–250 m*l* sterilized natural seawater adjusted to 30 ppt with glass-distilled water. The water was changed every 14–21 days as indicated by the general vigor of the plants. These vessels were held at 23–25 °C, photon fluence rate (PFR <10 μmol $m^{-2}s^{-1}$, and variable daylength of 12–16 h day^{-1}. As the plants adjusted to laboratory conditions, clean actively growing individual branches (5–10 mm long) were excised and isolated into 50×70 mm crystallizing dishes (Pyrex 3140) containing 50–75 m*l* 10% normal-strength Provasoli's Enriched Seawater (PES) (sterile 30 ppt seawater with 2 m*l* PES per liter) as specified by MCLACHLAN (1973) except that TRIS buffer was eliminated to reduce bacterial growth.

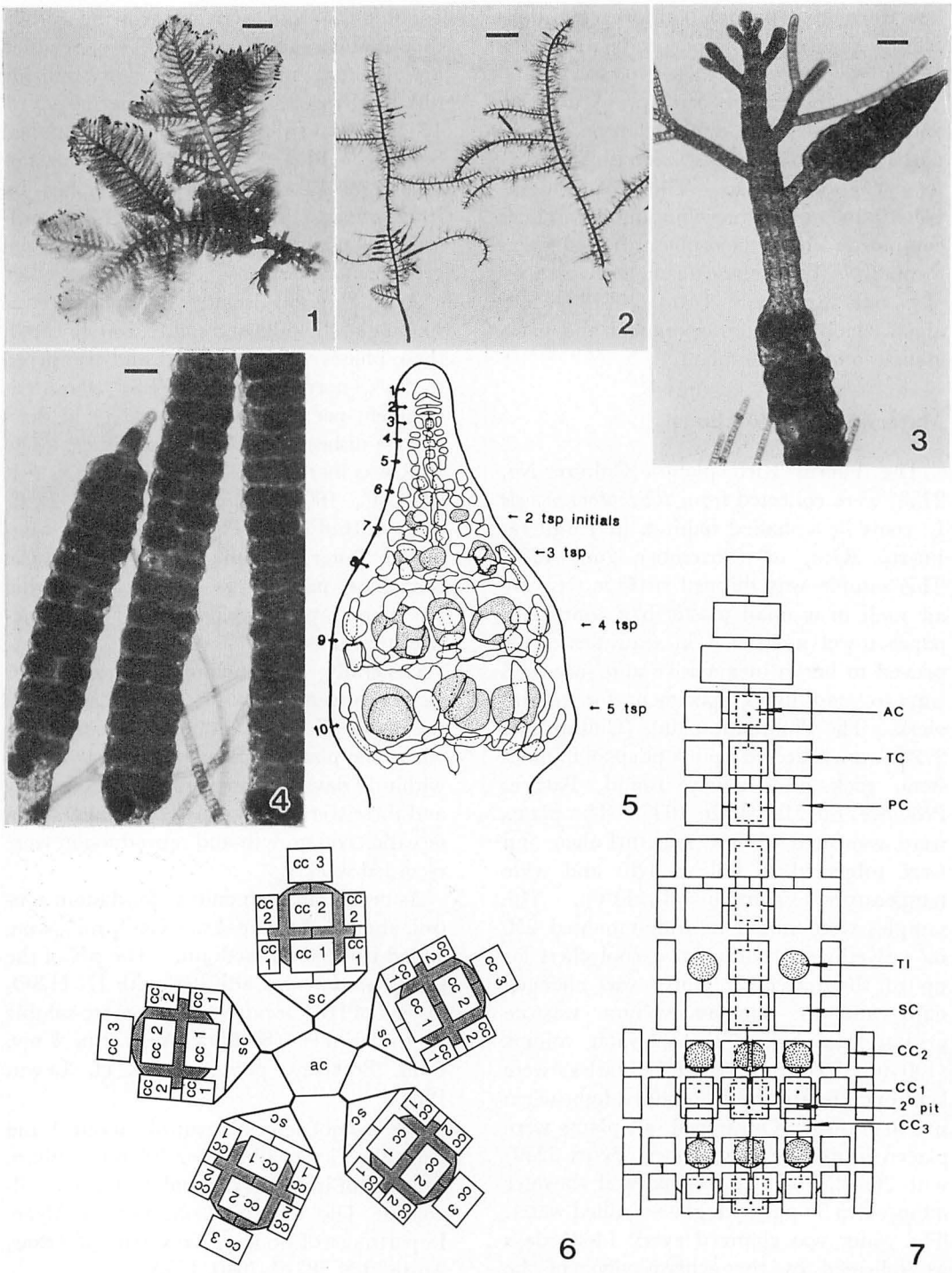
As the initial isolates produced tetrasporangial stichidia and spores were released, these phases were separated and transfered to 25% normal-strength PES (10 m*l* enrichment per liter sterile seawater) in deep storage dishes and 50×70 mm dishes. The PFR was increased to 30–50 μmol $m^{-2}s^{-1}$ at 23–25°C, 100–150 μmol $m^{-2}s^{-1}$ at 27°C, both at 16:8 LD. The latter regime stimulated faster growth and more natural branching patterns as well as enhancing the more rapid development of reproductive structures.

Generally, the medium and container were changed every 14–21 days except at the higher PFR and temperature conditions where the plants became pale yellow-brown within 14 days because of nitrogen deficiency and these were changed every 7 days. Observations on growth and reproduction were recorded weekly.

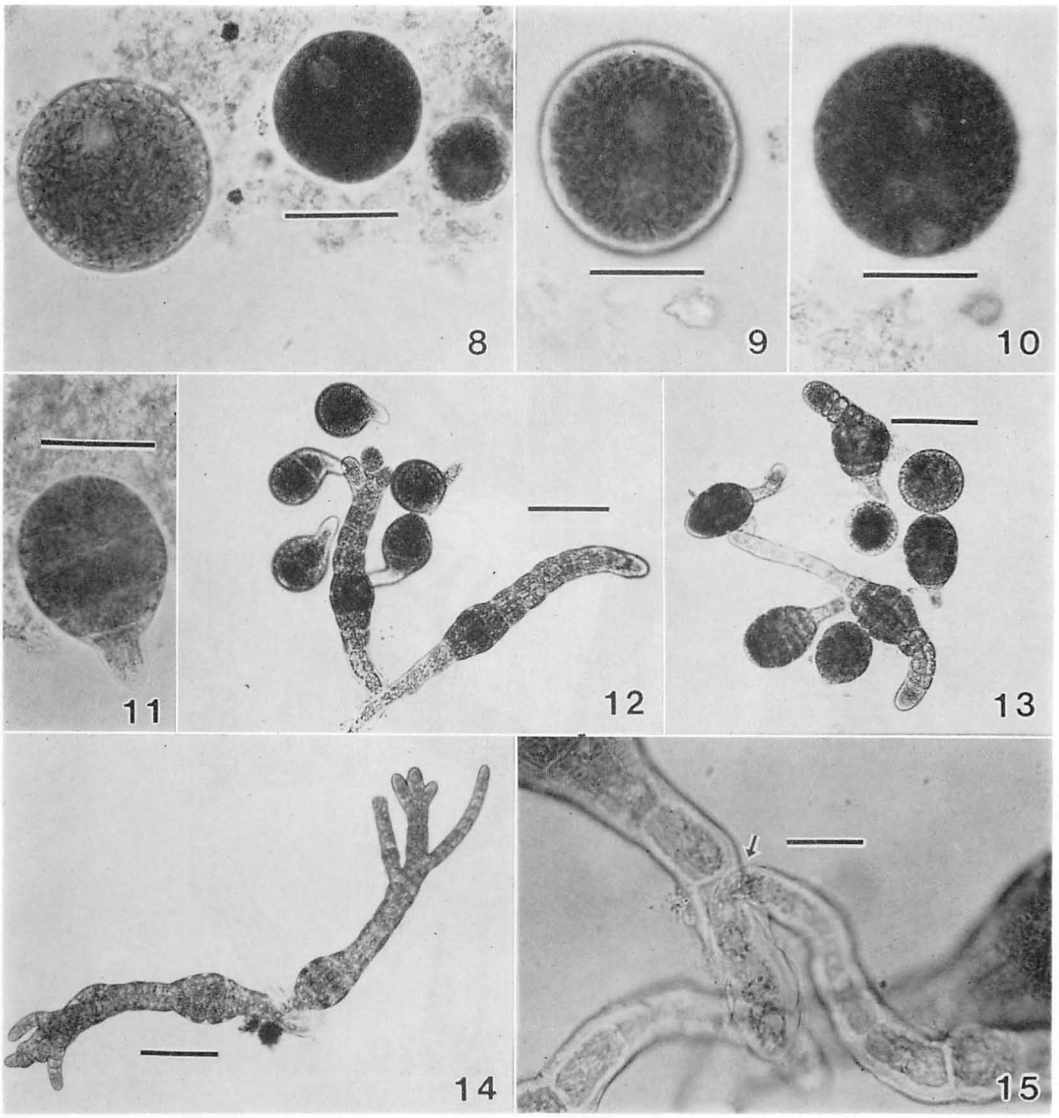
As needed in each culture for diatom control, about 4 drops of 1 mg GeO_2 ml^{-1} were added per 200 m*l* medium. The pH of the GeO_2 solution was adjusted with 1N H_2SO_4 instead of HCl because GeO_2 is more soluble in the former (Barbara WAALAND, Univ. Calif. Berkeley, pers. comm., cf. LEWIN 1966).

For cyanobacterial control, about 1 mg Penicillin G was added per 100 m*l* medium. Cultures of isolates 2722 and 2756 are available at The Culture Collection of Algae, Department of Botany, University of Texas, Austin TX 78713–7640, USA.

Photographic and ink illustrations were made from either living plants or those fixed in 5% formalin-seawater. Fixed specimens were rinsed in distilled water 5–30 min,

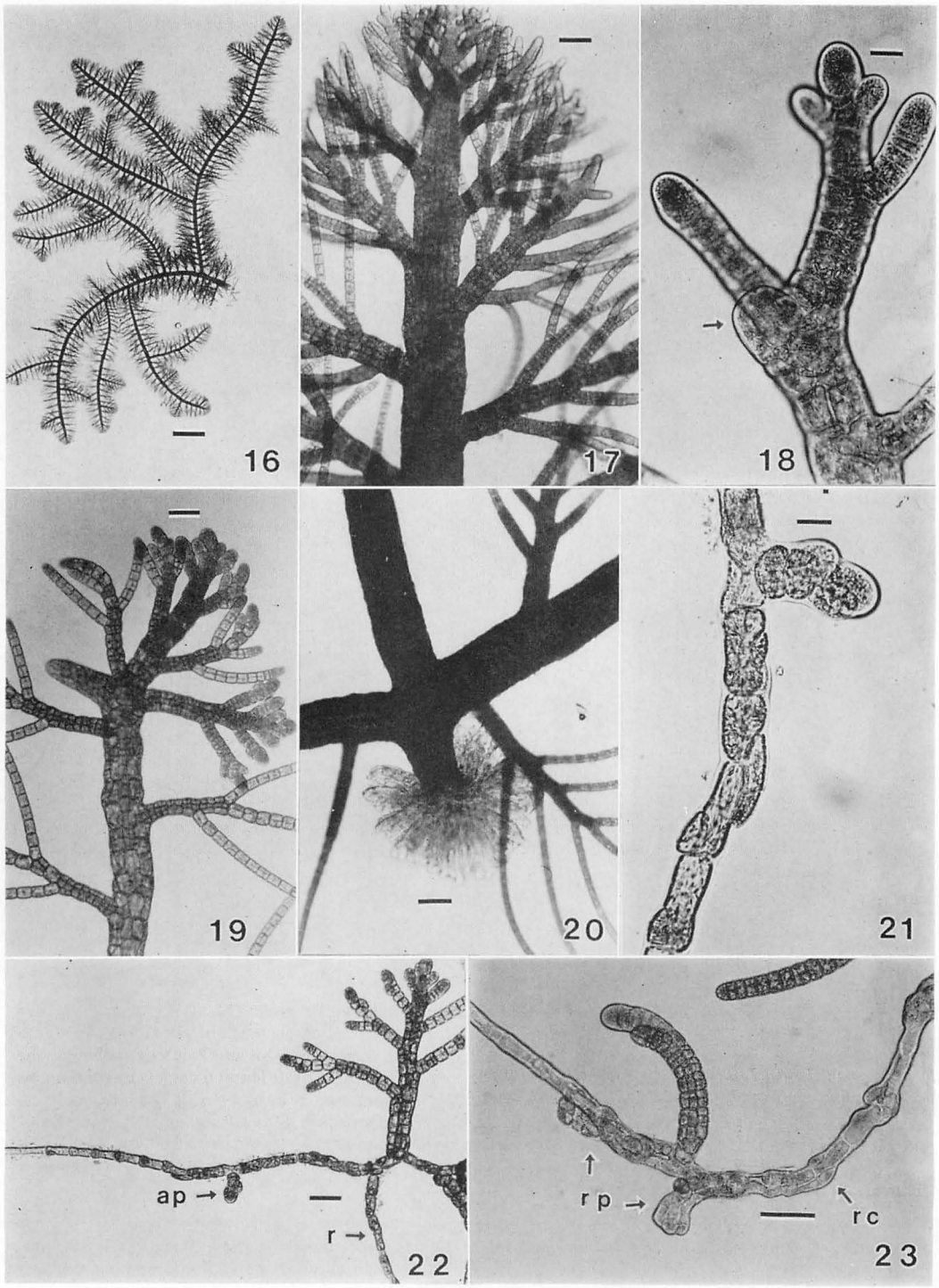


Figs. 1–7. Tetrasporophyte and tetrasporangia of *Bostrychia tenella* (LAM.) J. AGARDH isolate 2722. 1. Habit of field collected tetrasporophyte. Tetrasporangial stichidia are at tips of branches. Scale bar is 0.3 cm. 2. Habit of laboratory cultured tetrasporophyte. Note the reduced cortication and branching frequency. Tetrasporongial stichidia scattered along main axis and laterals. Scale bar is 0.3 cm. 3. Apex of stichidium that reverted to vegetative growth and formed a secondary stichidium at right. Scale bar is



Figs. 8–15. Tetrasporores, carpospores and sporelings of *Bostrychia tenella* (LAM.) J. AGARDH isolates 2722 and 2756. 8. 2722-Uninucleate tetraspores of different diameters on left and right. Center spore is binucleate. Scale bar is 50 μm. 9. 2722- Quadrinucleate tetraspore with one nucleus visible at one focal level. Scale bar is 50 μm. 10. 2722-Same tetraspore as Fig. 9 but at different focal level with remaining three nuclei visible. Scale bar is 50 μm. 11. 2722-Germinating tetraspore with rhizoidal pole established at 5-celled stage. Scale bar is 50 μm. 12. 2756-Tetraspores in various germination stages. Scale bar is 100 μm. 13. 2722-Carpospores in various germination stages. Scale bar is 100 μm. 14. 2756-Carpospore germlings with fused basal cells. Scale bar is 100 μm. 15. 2756-Rhizoids of carpospore germlings fusing together (arrow). Scale bar is 25μ m.

100 μm. 4. Three stichidia of varying lengths with 14 to 25 sporangial whorls. Scale bar is 100 μm. 5. Drawing of developing stichidium showing axial cell placement and number on left and sporangial placement and numbers on right. 6. Diagrammatic cross section of tetrasporangial whorl showing the axial cell (ac), tetrasporangial supporting cell (sc), sporangia (dark circles) and cover cell (cc) formation sequence. 7. Diagrammatic longitudinal section of stichidium showing axial cell (ac), pericentral cell (pc), tier cell (tc), supporting cell (sc), tetrasporangial initial (ti) and cover cell (cc) position, and secondary pit (2° pit) between cover cells of adjacent sporangial whorls.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softened in saturated chloral hydrate for 5–15 min, rinsed in distilled water for 5 min and mounted in 65% Karo syrup in distilled water with 0.04% anilin blue-black in 0.1% acetic acid (5% formalin was added to prevent fungal growth in the mounting medium). Coverslips were sealed with clear nail-varnish.

Observations

Bostrychia tenella 2722 Philippines

Tetrasporophytes

This isolate shows basically the same growth pattern in laboratory culture as in the field, i.e., dorsiventral orientation, alternate pinnate determinate branches and alternate indeterminate branches at uniform intervals associated with the ventral flagelliform haptera (Figs. 16–19). However, the frequency and length of branching as well as cortical development are reduced in laboratory cultured plants (cf. Figs. 1–2, 19). The main axial branches are polysiphonous with 6–7 pericentral cells and heavily corticated. Each determinate branch is polysiphonous (4 pericentral cells) and corticated except for the terminal 4 cells and bears 4–6 alternate branches that are polysiphonous (4 pericentral cells) on the basal segments and monosiphonous in the upper 20–30 cell segments. Often, the ultimate monosiphonous filaments are branched.

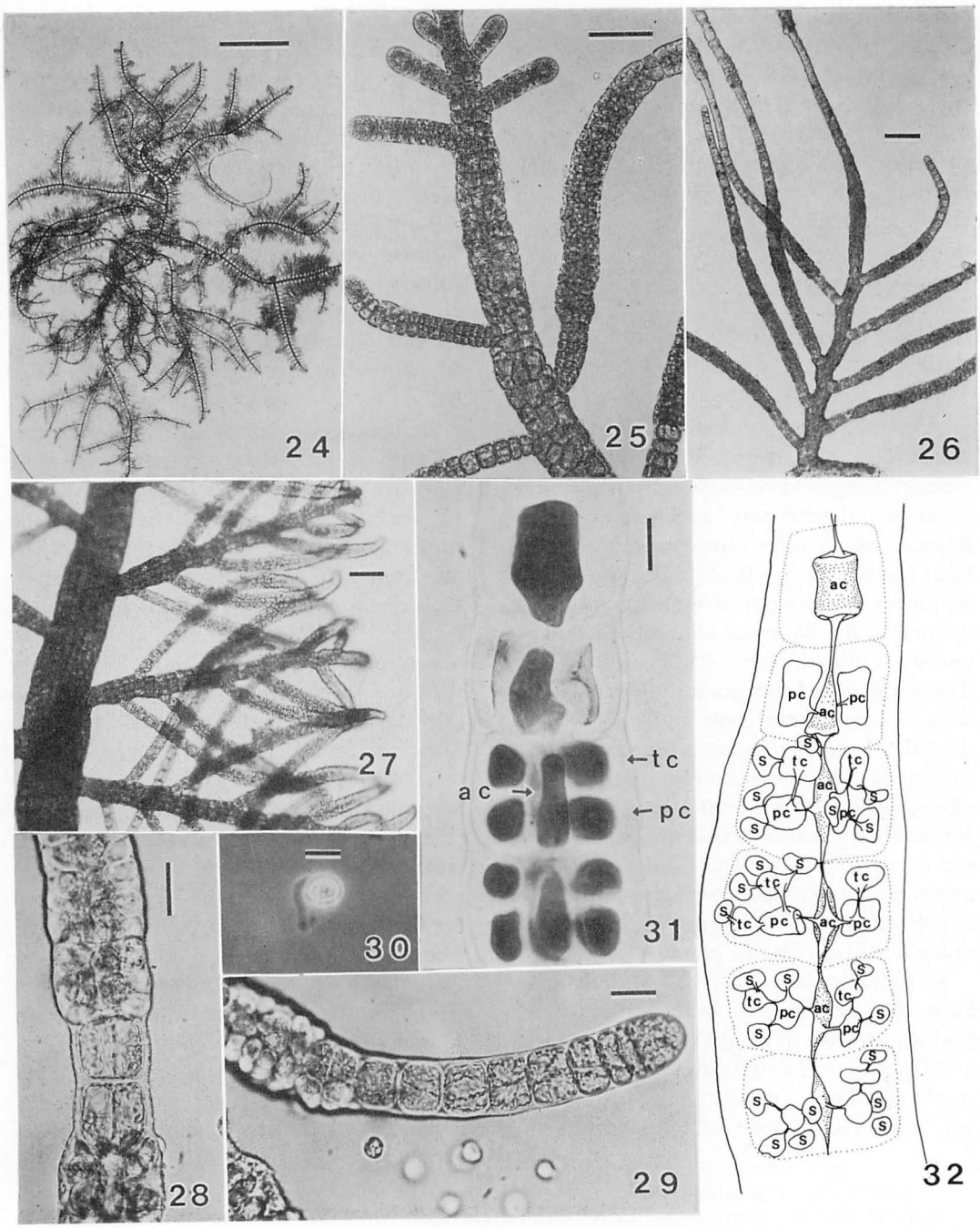
Tetrasporangial stichidia differentiate from the determinate branches and are conical, variable in length (mean=2.0 mm; n=20; range=0.2–5.0 mm) and diameter (mean=200 μm; n=20; range=180–250 μm) with 4–50 whorls of tetrahedral sporangia that are 70–85 μm in diameter when fully developed (Fig. 4). Normally, 4–5 mature sporangia develop per segment but it is not uncommon to see 1, 2 or 3 mature sporangia per whorl (Fig. 4), with either no other sporangia or with small incompletely divided abortive sporangia present. Some stichidia continue to grow indefinitely, forming new sporangial whorls at the apex. Others on the same plant revert to vegetative growth, establishing a new series of lateral indeterminate and determinate branches that later form new stichidia (Fig. 3).

In sporangial development, five pericentral cells are cut off 4–8 axial cells from the apex (Fig. 5). Each pericentral cell then divides to form the tetrasporangial initial on the upper face. Next, three cover cell initials are formed from the lower cell, which is now the supporting cell, and these divide further. The lateral cover cell initials form one secondary cover cell each (sometimes two) and the central cover cell cuts off two secondary cover cells upward. In some cases, the central cover cell appears to cut off a secondary cover cell from the lower face and the other is cut off the upper face (Fig. 6). By the 10th whorl from the apex, secondary pit connections are seen between adjacent cover cells in the same whorl and between cover cells derived from adjacent whorls above and below (Fig. 7).

Vegetative development of tetraspore germlings

The tetraspores vary in diameter between 36 and 76 μm (mean=58 μm; n=15) (Fig. 8). Most appear uninucleate although this is difficult to determine because of the densely packed chloroplasts. Some larger spores

Figs. 16–23. Vegetative plants of *Bostrychia tenella* (LAM.) J. AGARDH isolate 2722. 16. Habit of vegetative plant in culture. Scale bar is 0.3 cm. 17. Dorsal view of main shoot apex showing monosiphonous laterals on determinate branches. Scale bar is 100 μm. 18. Indeterminate lateral branch with hapteron developing (arrow) at junction with secondary and indeterminate branch. Scale bar is 50 μm. 19. Indeterminate lateral branch with hapteron and secondary indeterminate branch in later development than Fig. 18. Scale bar is 100 μm. 20. Hapteron with adhesive tip filaments formed in contact with the substrate. Scale bar is 100 μm. 21. Basal monosiphonous rhizoidal filament with secondary erect axis primordium above and lenticular cells below. Scale bar is 50 μm. 22. Rhizoidal filament with one axial primordium (ap), a branched secondary axis and a secondary rhizoid (r). Primary axis base at far right. Scale bar is 100 μm. 23. Rhizoidal filament with corticating cells (rc) and two adhesive pads (rp). Scale bar is 100 μm.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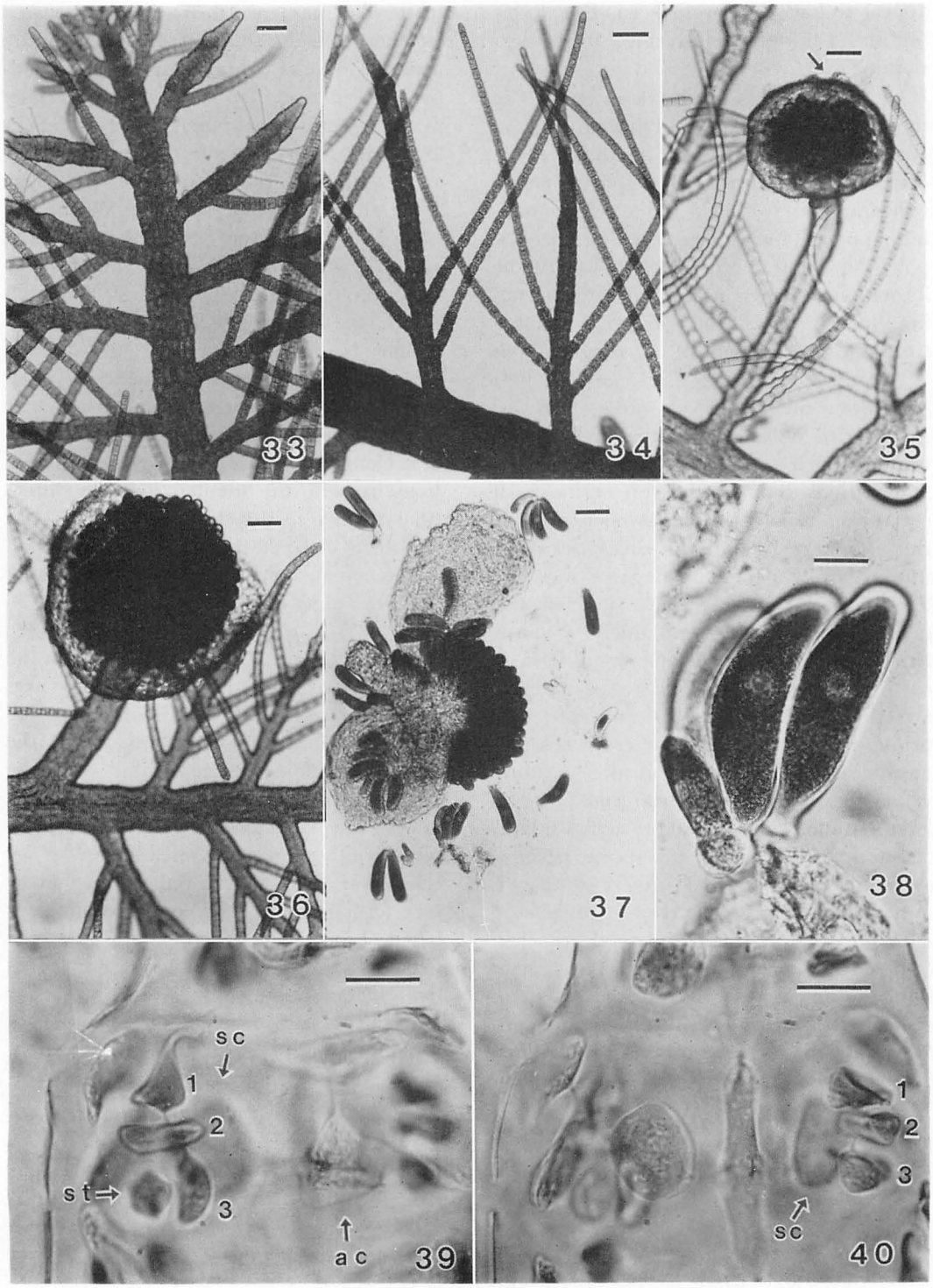
appear to have 2, 3 or 4 nuclei (Figs. 8–10) but this has not been verified by nuclear staining.

Generally, the tetraspores germinate in the manner illustrated by PRUD'HOMME VAN REINE and SLUIMAN (1980) for *B. scorpioides* (HUDSON) MONTAGNE. The spores first divide transversely 2 or 3 times before establishing polarity with formation of a rhizoidal pole (Fig. 11) that is quickly followed by the formation of the erect axis when the germling is about 10 cells long (Fig. 12). Sometimes the development of the rhizoidal axis is slower at lower PFR ($<15\ \mu mol\ m^{-2}s^{-1}$) and lower nutrient levels (10% PES) or in crowded cultures, resulting in the failure to attach to the substrate. The first longitudinal divisions develop at the 5–10 cell stage, usually in the swollen area of the original spore and these divisions extend upward as the erect axis elongates (Fig. 12). The rhizoidal cells continue to elongate, divide transversely or obliquely to form lens-shaped cells that elongate to form a corticating cell layer (Fig. 23rc), specialized attachment cells (Fig. 23rp) and erect axis primordia (Fig. 22ap). The erect axis primordia are well defined and after 3 transverse divisions the subapical cell forms a lateral branch (Fig. 21) and the longitudinal cells of the polysiphonous sector become evident. When the main axis is less than 1 mm long there are usually 5–6 (range=2–10) compound determinate lateral branches evident. At a point about 10–12 axial cells from the apical cell, several adjacent pericentral and cortical cells on the ventral surface elongate in unison to form the first flagelliform hapteron (Figs. 18–19). This pattern of hapteron formation is repeated at about 1–2 mm intervals along the entire axis. The lateral branch immediately adjacent and dorsal to the hapteron usually is an indeterminate branch. These branches are formed repeatedly on alternate sides of the axis and grow upward to form a new indeterminate axial system. When the elongating hapteron establishes contact with the substrate, a discoid multicellular adhesion pad (Fig. 20) forms. The position of the haptera on the axis is fixed and cannot be reversed even by subsequently growing the plant in an inverted position. The haptera exhibit neither a geotropic nor phototropic response and as the axial system elongates and rotates in various directions haptera are oriented in many directions relative to the substrate.

Growth is somewhat slow and the main axes reach an average of about 3.0 mm long (1.9–3.7 mm) after 45 days (at PFR>30 $\mu mol\ m^{-2}s^{-1}$, 25°C, 25% PES) when there are about 3 haptera per axis. When the main axis is 2–3 mm long, secondary axes already are well developed from either the basalmost indeterminate branch or the basal rhizoidal system. Plants in close proximity often adhere together by growth of rhizoids or haptera. This is accomplished either by cell wall adhesion or by cell fusion (Figs. 14–15) through secondary pit formation. These observations are based on 50 plants in one 50×70 mm dish with 75 m*l* 25% PES medium. Reproductive differentiation generally does not occur until the plants become about 1.0 cm long.

Cultures were started on May 27th, 1986. The original tetrasporophytes pro-

Figs. 24–32. Male gametophytes and spermatangia of *Bostrychia tenella* (LAM.) J. AGARDH isolate 2722. 24. Habit of 1-yr old male gametophyte in culture. Scale bar is 1 cm. 25. Enlarged view of apex of Fig. 27 showing the polysiphonous stichidium on right. Scale bar is 100 μm. 26. Monosiphonous spermatangial stichidia on lateral determinate branch excised from male gametophyte. Scale bar is 100 μm. 27. Polysiphonous spermatangial stichidia on determinate branches. Scale bar is 100 μm. 28. Subapical segment of monosiphonous stichidium with developing spermatangia above. Scale bar is 25 μm, 29. Apical segment of monosiphonous stichidium with discharged spermatia below. Scale bar is 25 μm. 30. Discharged spermatium with excreted polysaccharide tail. Phase contrast. Scale bar is 5 μm. 31. Subapical segment of stichidium stained with aniline blue black showing pericentral cell (pc) and tier cell (tc) formation. Scale bar is 100 μm. 32. Drawing of stichidium illustrating axial cells (ac), pericentral cells (pc), tier cells (tc) that serve as spermatangial initials, and spermatangia (spm).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duced functional tetrasporangia and the tetraspores germinated into 1) male gametophytes with spermatangia, 2) female gametophytes with procarps, 3) bisexual gametophytes with spermatangia, procarps, and carposporophytes, and 4) mixed-phase plants with spermatangia, procarps, carposporophytes, and abortive tetrasporangia, which were observed on March 1st, 1987. Carpospores were released and isolated from gametophytes on April 1st; these did not develop into tetrasporophytes but into gametophytes again, namely 1) male gametophytes with spermatangia, 2) female gametophytes with procarps and carposporophytes, 3) bisexual gametophytes with spermatangia, procarps, and carposporophytes, and 4) mixed-phase plants with spermatangia, procarps, carposporophytes, and abortive tetrasporangia, which were observed on September 15th, 1987.

Male gametophytes

Male plants become reproductive earlier and remain 1/3–1/2 smaller throughout their life than the bisexual, unisexual and mixed-phase plants. This pattern is also evident in other *Bostrychia* species (WEST and CALUMPONG, unpubl.) as well as many other red algae, and is presumably because a greater biomass is contributed to reproduction in males than in other phases.

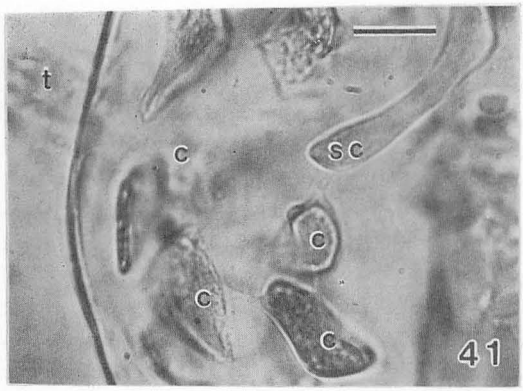
Spermatangia

In male plants grown under optimal conditions, it appears that more than 50% of the determinate branch surface area is covered with spermatangia because both the polysiphonous (Figs. 25–27) and the monosiphonous (Fig. 26) ultimate filaments become reproductive. At variable distances (3–10 cells) from the branch apex (Fig. 29), four pericentral cells develop from each axial cell. In higher PFR, the pericentral cells are formed closer to the apex than in lower light. Each pericentral cell then forms 1–2 tier cells that give rise to 2–3 spermatangial initials (Fig. 32). In living specimens, the outer spermatangial layer appears very pale to colorless whereas the underlayer of spermatangial initials and axial cells are dark red. In anilin blue-black, axial cells, pericentral cells and tier cells stain purple (Fig. 31) whereas the spermatangia stain light blue. The spermatangial sectors are variable in length (1–12 axial cells) often with monosiphonous (1–4 cells long) portions between spermatangia sectors (Fig. 26). When an axial cell of the monosiphonous or polysiphonous sector dies, a new cell grows upward through the dead cell reforming the axial continuity.

As with other *Bostrychia* species observed in culture, the spermatia of one sector are released almost stimultaneously *en masse* (WEST and CALUMPONG, unpubl.). Each spermatium is 7–9 μm diam with short (4–10 μm long) polysaccharide tail at one end (Fig. 30) similar to that observed in other Ceramiales (FETTER and NEUSHUL 1981; MAGRUDER 1984). The nucleus is evident as an oblate sphere (3 μm diam), slightly displaced by a large polysaccharide vesicle. The granular peripheral cytoplasm contains a faintly visible small pink chloroplast. The released spermatia adhere only to the trichogynes of the female procarps (Fig. 59).

Some male plants remain unisexual throughout their life continuing to grow and reproduce indefinitely (e.g., Fig. 24). Others become bisexual with separate procarpic stichidia or they develop mixed-phase reproduction with stichidia bearing

Figs. 33–40. *Bostrychia tenella* (LAM.) J. AGARDH isolate 2722 female gametophyte. 33. Axis with stichidia and numerous procarps. Scale bar is 100 μm. 34. Axis with stichidia and sparse procarps. Scale bar is 100 μm. 35. Mature cystocarp with ostiole (arrow). Scale bar is 100 μm. 36. Mature cystocarp with pericarp broken away near apex exposing carposporangial mass. Scale bar is 100 μm. 37. Ruptured cystocarp with about 50 carpospores visible. Scale bar is 100 μm. 38. Undischarged carpospore with central nucleus. Scale bar is 25 μm. 39. A pair of procarps on the fifth axial cell. The left procarp has a supporting cell, 3 cells and a sterile cell. Cf. with Fig. 45. Scale bar is 10 μm. 40. At a different focal level from Fig. 39, the supporting cell of the left procarp is evident and the 3-celled carpogonial branch on the right is in focus. Cf. with Fig. 45. Scale bar is 10 μm.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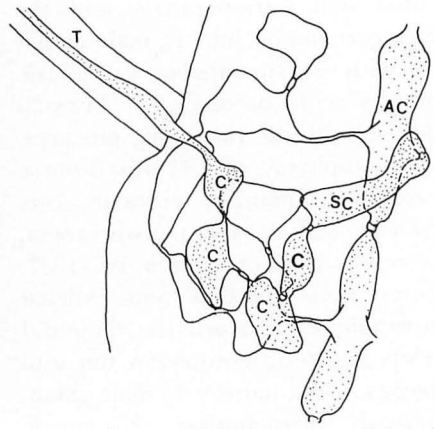
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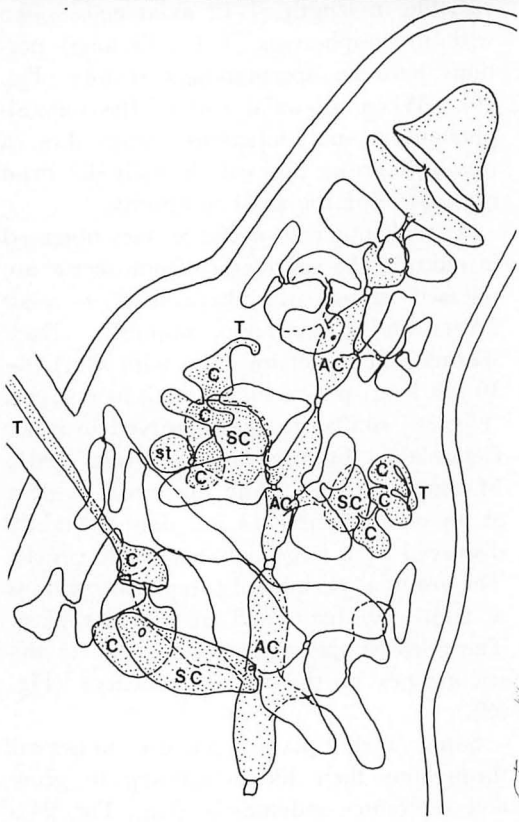
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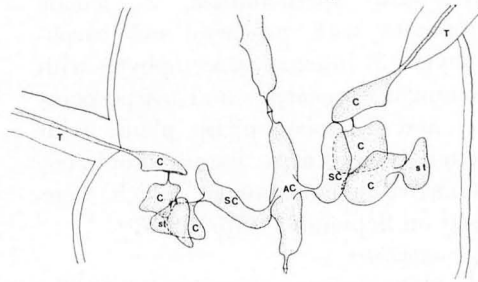
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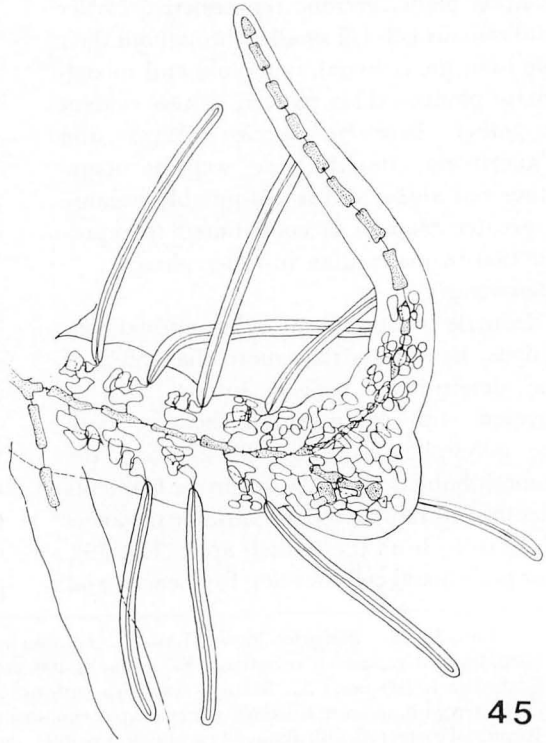
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both functional procarps and abortive tetrasporangia.

Female gametophytes

In laboratory culture with unisexual and bisexual gametophytes present procarps usually do not develop as soon on the female gametophytes (>1.0 cm) as do the spermatangia on male gametophytes. They appear on the determinate polysiphonous laterals (Figs. 33–36) borne in the same manner as spermatangial and tetrasporangial stichidia on the other phases. Individual female gametophytes isolated in separate containers initiate procarps at an earlier stage (<5 mm long) and continue to produce procarps throughout the life of the plant (>12 months). The density of procarps on stichidia of isolated females is often greater than on those grown in cultures with males and bisexual plants (cf. Figs. 33 and 34) although procarp frequency on stichidia is variable. The procarpial stichidia begin to differentiate from the 5–10th determinate branch on the main axis (Fig. 33). Each stichidium is clavate (0.5–1.0 mm long) and develops between 5–10 procarps initially, but when they are not fertilized the stichidia exhibit indeterminate growth becoming up to 1 cm long (with 50 or more procarps) and developing secondary vegetative branches and new procarpial stichidia. Trichogynes are 4–5 μm in diam and 30–120 μm long with a continuous homogeneous protoplast and a swollen tip. In older unfertilized trichogynes the protoplast contracts to form a thin strand and they commonly are broken off near the base. Adhesion of spermatia is evident even in the short developing trichogynes. Procarps first become evident on the 5–7 th axial cell of the stichidium (Fig. 43). Each axial cell produces 4 pericentral cells and one or, more frequently, two procarps are formed on each sector (Figs. 39–40, 43–45).

Bisexual and unisexual female plants as well as mixed-phase plants bear carpogonial branches that are 2-, 3- or 4-celled with one sterile cell or none (Figs. 39–43). Three-celled carpogonial branches are more common than 2- or 4-celled carpogonial branches.

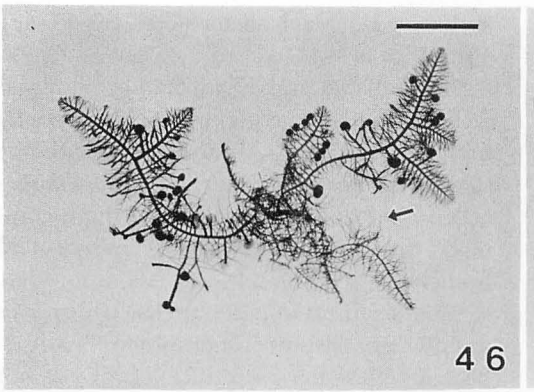
After fertilization, the pericarp develops rapidly as the carposporophyte enlarges. Because of the pericarp thickness, it was not possible to see post-fertilization stages clearly. Mature cystocarps are about 415 μm mean diam (range=350–495 μm; n=30). A well defined ostiole is formed about midway through cystocarp development. Fully developed ostioles are 100–150 μm in diameter (mean=120 μm; n=20). Near their bases, mature cystocarps develop adventitious monosiphonous or polysiphonous branches that form prcarps. The upper pericarp of some cystocarps fractures leaving the mature carpospores fully exposed (Fig. 36).

Attached carpospores do not germinate *in situ*. Carposporangia are elongate and clavate (100–175 μm long and 35–45 μm diam) with a single spherical nucleus (12–15 μm diam) (Fig. 38). About 75–130 carpospores are released from each cystocarp over 3–6 days. Discharged carpospores are spherical and 55–65 μm diam. Carpospores exhibit the same pattern of germination and differentiation as the tetraspores (cf. Figs. 13–15 with Fig. 12) but develop into bisexual and unisexual gametophytes and mixed-phase plants.

Bisexual gametophytes and mixed-phase plants

Generally in bisexual plants, spermatangial sectors are seen on determinate branches spatially separated from those bearing procarps, however, there are ex-

Figs. 41–45. Procarps of *Bostrychia tenella* (LAM.) J. AGARDH isolate 2722. 41. Four-celled carpogonial branch and supporting cell. Carpogonium and trichogyne (t) out of focus. Scale bar is 11 μm. 42. Drawing of 4-celled carpogonial branch in Fig. 41. 43. Drawing of developing procarps on the 5th axial cell as in Figs. 39–40. 44. Drawing of axial cell (ac) with two supporting cells and two 3-celled carpogonial branches with one sterile cell each. 45. Drawing of stichidium with monosiphonous apex, carposporophyte developing on the 13th axial cell, and 3-celled carpogonial branch on the 15th through the 18th axial cells.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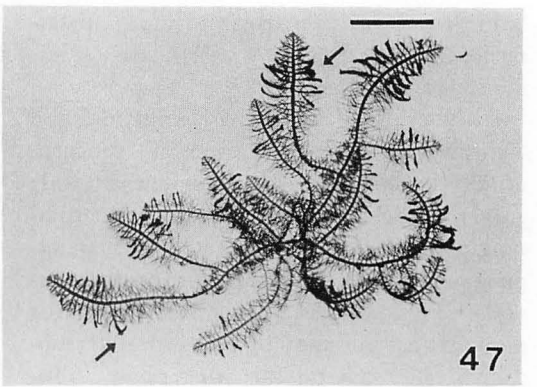
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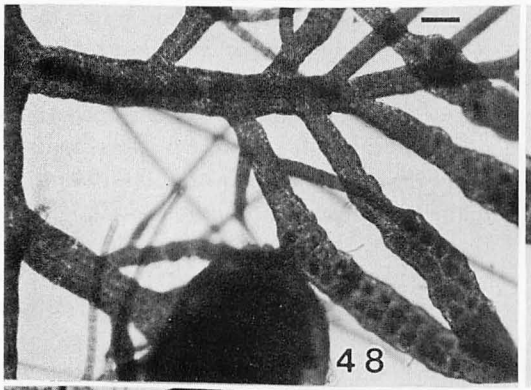
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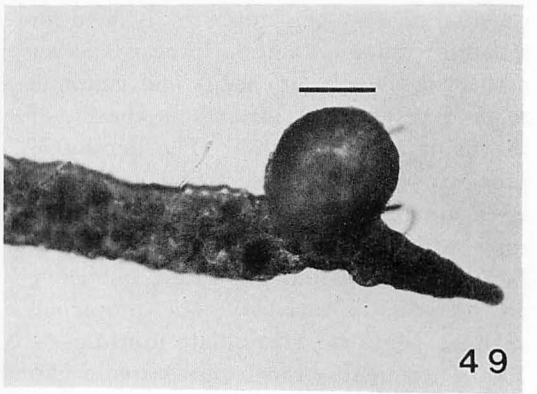
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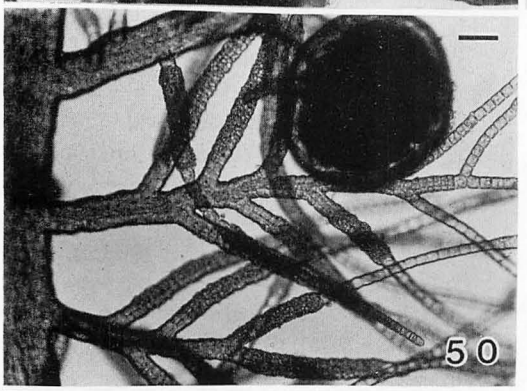
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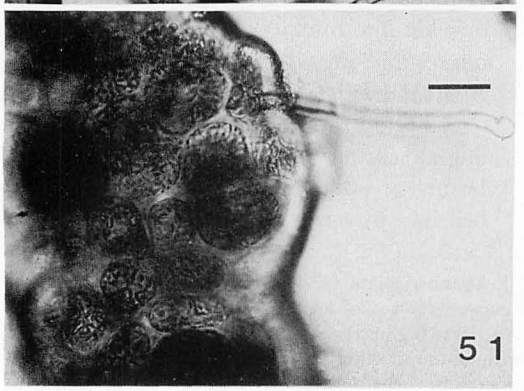
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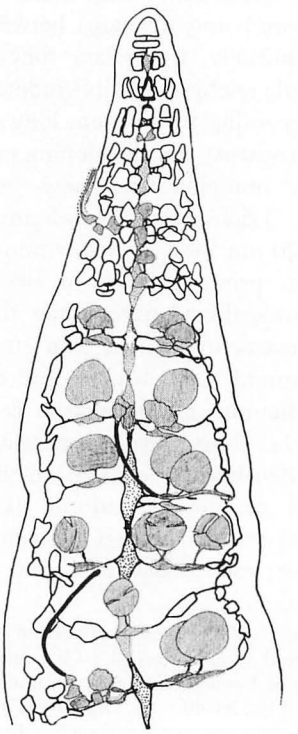
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ceptions (Fig. 50). These spermatangia/-procarp-bearing stichidia usually do not develop cystocarps although branches with only procarps present formed cystocarps readily.

Mixed-phase plants derived from tetraspores exhibit no abormal growth and are usually as large as or sometimes larger than other phases. Generally, these mixed-phase plants develop spermatangia on different stichidia than the procarps and tetrasporangia (Figs. 46, 47, 50). On procarpial/tetrasporangial stichidia, procarps are often borne on the same whorl as tetrasporangia (Figs. 51–52). Frequency of cystocarp formation is variable (cf. Figs. 46 and 47) and they may develop on either procarpial stichidia or procarpial/tetrasporangial stichidia (cf. Figs. 48, 49 and 50). The cystocarps all appear functional, releasing viable carpospores that germinate into unisexual and bisexual gametophytes and mixed-phase plants. No tetrasporophytes are formed and no plants with only spermatangia and tetrasporangia are seen. Individual mixed-phase plants maintained in separate cultures continued to show the same reproductive pattern for many months.

The 2722 original tetrasporophyte continues to produce fully functional tetrasporangia and the tetrasporse also germinate into unisexual, bisexual and mixed-phase plants.

Bostrychia tenella 2756 Puerto Rico

Tetrasporophytes

Bostrychia tenella 2756 Puerto Rico retained the general vegetative features of the field-collected plants. The determinate branch axes are polysiphonous except for the last 5–10 cells whereas the determinate branch laterals are monosiphonous except for the basal cell. KUMANO (1979, Figs. 30 and 20) illustrates this difference between *B. tenella* and *B. binderi* HARVEY quite well. The vegetative growth and development of 2722 and 2756 appear quite similar and the basic differences lie in the reproductive pattern of the tetraspore germlings.

Cultures were started on March 3rd, 1987. The tetraspores, which were isolated from a tetrasporophyte germinated into 1) male, 2) female, and 3) bisexual gametophytes with carposporophytes, which were observed on September 20th and October 31st, 1987.

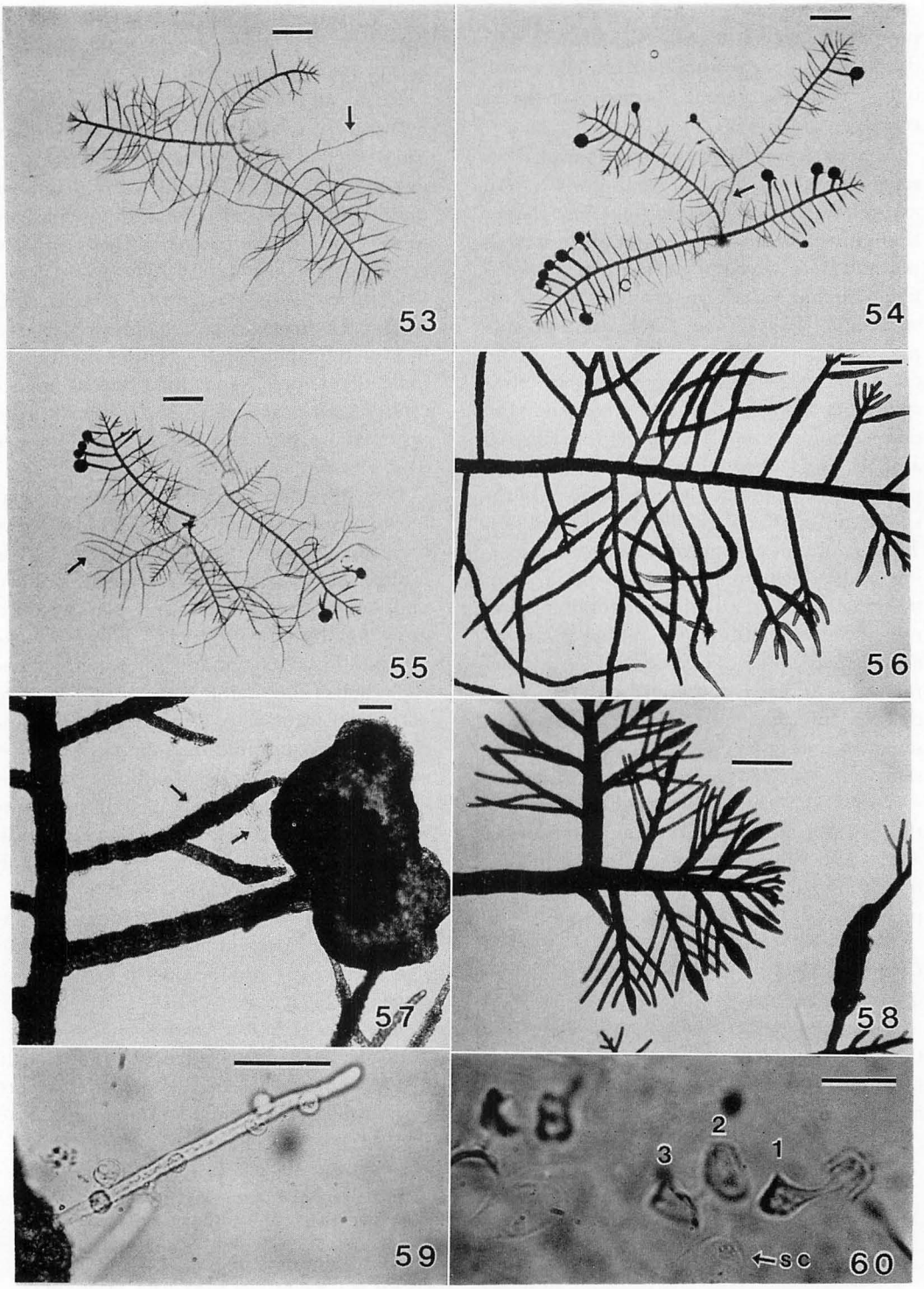
It is important to note that the original tetrasporophyte isolate of 2756 did bear one male branch with spermatangia. This branch was isolated into a separate culture and, up to the present, has continued to grow vigorously, producing only spermatangial branches. Likewise, the tetrasporophyte has also maintained its reproductive pattern in forming only tetrasporangial stichidia. It is possible that the male branch resulted from *in situ* germination of a tetraspore in the tetrasporophyte but no tetrasporangia were observed on the plant until nearly three months after the male branch developed.

The tetrasporophyte of 2756 develops numerous tetrasporangial stichidia (Fig. 58). that are similar in all respects to those observed in 2722.

Vegetative development of tetraspore germling

Discharged tetraspores are all uninucleate and develop as seen in Fig. 12. Tetraspore discharge and germination are similar to

Figs. 46–52. *Bostrychia tenella* (LAM.) J. AGARDH isolate 2722 mixed-phase plants derived from tetraspores. 46. Mixed-phase plant with numerous cystocarps on procarpial/tetrasporangial stichidia. Male branches in lower part (arrow). Scale bar is 0.5 cm. 47. Mixed-phase plant with numerous procarpial/tetrasporangial stichidia and a few developing cystocarps (arrows). Scale bar is 0.5 cm. 48. Several procarpial/tetrasporangial stichidia on determinate branch. Tetrasporangia are abortive. Mature cystocarp on adjacent lower procarpial stichidium. Scale bar is 100 μm. 49. Developing cystocarp on procarpial/tetrasporangial stichidium. Scale bar is 50 μm. 50. Procarpial stichidium with mature cystocarp. Two lower branches bear spermatangia. Scale bar is 100 μm. 51. Emergent trichogyne of procarp borne in whorl with two tetrasporangia. Scale bar is 25 μm. 52. Drawing of procarpial/tetrasporangial stichidium with abortive tetrasporangia and 3- and 4-celled procarps.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those of 2722 except that 2756 gametophytes develop faster and reproduce sooner than those of 2722 and do not show mixed-phase reproduction. The average length of the tetraspore-derived mature plants in 2722 is between 20 and 30 mm whereas those of 2756 are 7–8 mm long (Figs. 53–55). In both isolates the male gametophytes are often smaller than the other reproductive plants including females, bisexual gametophytes and mixed-phase plants. This size difference is considered also in the section on male gametophytes of 2722.

It is also evident that some gametophytes derived from tetraspores of 2756 and 2722 that are initially unisexual do become bisexual with increasing age. This is quite common in other *Bostrychia* species (WEST, unpubl.). Usually the females first produce spermatangial stichidia on the lower branches and later on the procarpic stichidia formed in the upper parts.

Isolated bisexual gametophytes develop cystocarps readily (Fig. 57), indicating that they are self-compatible, a trait that is observed in most bisexual red algae in culture.

Unisexual male gametophytes (Figs. 53, 56) appear to become bisexual less often than females but longer-term investigations of a larger number of developing gametophytes are necessary to fully assess this process. Of 74 plants initially counted, there were 42% females, 24% males, 32% bisexual plants and 2% vegetative plants but after another 40 days, the females represented 43%, males 29% and bisexual plants 28% of the 120 plants.

The carpogonial branches of 2756 bisexual and uniseuxal gametophytes are 2-, 3- or 4-celled with one sterile cell or none. The 3-celled carpogonial branches are most common (Fig. 60). Either one or two procarps are formed on each axial cell.

Carpospores derived from bisexual and unisexual female gametophytes developed into more gametophytes after 3 months. The first reproductive plants to appear were unisexual males 2–4 mm long. Females and bisexual plants developed later but not tetrasporophytes. Further development of these plants is being monitored.

Discussion and Conclusions

Although *Bostrychia tenella* is widely distributed in warm temperate to tropical habitats, tetrasporangia are usually the only reproductive structures observed (e.g. BØRGESEN 1918). CHAPMAN (1960) and TAYLOR (1960) mention tetrasporangia and cystocarps but procarps and spermatangia are not noted. It is, therefore, interesting that the sexual structures are so conspicuous in continually immersed culture. In their natural habitats of either rock or mangrove the plants are subjected to long periods of exposure, extreme temperatures and wide ranges of salinity. Continuous immersion culture as described here represents an atypical environment for *Bostrychia*. The conditions of light, temperature, nutrients, salinity and gas exchange are much more stable than those to which it is subjected in nature. Perhaps this alters the development, growth and reproduction somewhat. It may be necessary to provide a simulated tidal exposure system to make a clear comparison of the growth and reproduction in these two different environments.

The unusual reproductive events in *Bostrychia tenella* described here have not been reported previously. Perhaps this is

Figs. 53–60. *Bostrychia tenella* (LAM.) J. AGARDH isolate 2756. 53. 170-day old male. Long branches are spermatangial. Scale bar is 1 mm. 54. 170-day old female with cystocarps and connecting rhizoidal system (arrow). Scale bar is 1 mm. 55. 170-day old bisexual gametophyte with cystocarps and long thin spermatangial branches (arrow). Scale bar is 1 mm. 56. Male plants with most branches bearing spermatangia. Scale bar is 400 μm. 57. 170-day old bisexual gametophyte with double cystocarp. Trichogynes (t) and spermatangia evident on center branch (arrows). Scale bar is 100 μm. 58. Tetrasporophyte developing stichidia at branch tips. At lower right, an older stichidium reverted back to vegetative growth. Scale bar is 400 μm. 59. Five spermatia adhering to trichogyne. Scale bar is 25 μm. 60. Mature 3-celled carpogonial branch with supporting cell. Scale bar is 10 μm.

primarily because sexually reproductive plants are not commonly seen in field collections. There is presumably some key environmental factor that causes the gametophytic phase to be so transient or, perhaps, they do not appear at all in some localities. One then wonders why the usual red algal reproductive features appear in culture isolates of *B. tenella*. MAGGS (1988) reviewed many aspects of reproductive variability in the Florideophycidae, particularly mixed-phase reproduction, occurence of bisexual thalli in normally unisexual species and direct development of gametophytes from carpospores.

As other isolates of *Bostrychia tenella* develop in culture, we can ascertain the extent of mixed-phase reproduction in this species-complex. Some isolates of two other *Bostrychia* species, *B. radicans* (MONTAGNE) MONTAGNE and *B. binderi* have been observed to exhibit mixed-phase reproduction in culture similar to that observed in the Philippine isolate (2722) while all isolates of *B. montagnei* HARVEY, *B. pinnata* TANAKA et CHIHARA, *B. moritziana* (SONDER ex KUETZING) J. AGARDH and some isolates of *B. binderi* show normal *Polysiphonia*-type life history patterns even in immersed cultures (WEST *et al.*, unpubl.).

Traditionally, the Ceramiales is regarded as an assemblage of entities with 4-celled carpogonial branches. Other authors on *Bostrychia* reproduction (CORDEIRO-MARINO 1979, HOMMERSAND 1963, TANAKA and CHIHARA 1984b, KING and PUTTOCK 1986) have reported the standard Ceramiales-type 4-celled carpogonial branch in *B. arbuscula* HARVEY, *B. kelanensis* GRUNOW in POST, *B. pinnata* and *B. radicans*. The 2-, 3- and 4-celled carpogonial branches on female, bisexual and mixed-phase gametophytes of *B. tenella* 2722 and 2756, therefore, represent a significant departure. It has not been possible to determine if the 2- and 3-celled carpogonial branches are functional but it is important to note that functional carposporophytes are formed on all three reproductive phases and result only from fertilization by spermatia from a male or bisexual plant. No carposporophytes developed on females or on mixed-phase (procarpial/tetrasporangial) plants cultured separately from males. The 2- and 3-celled carpogonial branches are clearly not early developmental stages because the carpogonia on most of these have fully elongate trichogynes and are often 20 or more axial cells away from the apical area where procarps mature and can be fertilized. It is clear that reproductive success of these 3-celled procarp-bearing plants is not impaired because carposporophytes are numerous and the carpospores develop normally. The basic difference from typical carpospore progeny is that these all produce gametophytes that are bisexual, unisexual or mixed-phase. This pattern is consistent through two generations involving hundreds of plants. Although tetrasporangia often develop fully and undergo karyo- and cytokinesis, they are always abortive at maturity, appearing as shrivelled off-colored protoplasts.

With respect to the nuclear cytology of these mixed-phase reproductive stages, further investigation is required.

Acknowledgements

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Further Notes

Since the submission of this manuscript in December, 1987, the second generation of the Peurato Rican isolate 2756 has reproduced. The carpospores derived from first generation gametophytes developed into 11 unisexual males (24%), 12 bisexual gametophytes (27%), 2 mixed-phase plants (one male with tctrasporangial stichidia and one bisexual gametophyte with tetrasporangial stichidia) and 20 tetrasporophytes (45%). Viable tetraspores are produced by the tetraporophytes but mixed-phase plants have abortive tetrasporangia.

WEST J.A. and CALUMPONG, H.P.: 室内培養におけるコケモドキ属（紅藻，イギス目）の mixed-phase reproduction I. コケモドキについて

プエルトリコ産の四分胞子体からの四分胞子は不動精子の存在下でのみ果胞子体を形成する両性，単性の配偶体に発達する。果胞子は配偶体に発達する。フィリピン産の四分胞子体からの四分胞子は両性，単性配偶体及び配偶体の procarpial stichidia 上に形成される non-functional な四分胞子嚢をもつ mixed-phase 体に発達した。両性，単性配偶体及び mixed-phase 体の造果枝は 2，3，4 個の細胞からなる。variable（成熟）しうる果胞子体は不動精子が存在すればプロカルプを生じている全ての植物体上に発達する。配偶体並びに mixed-phase 体からの果胞子は四分胞子体には発達せず，再び両性，単性配偶体及び mixed-phase 体に発達する。(Department of Botany, University of California, Berkeley, California 94720)

第13回国際海藻シンポジウムについて

第13回国際海藻シンポジウム（The XIIIth International Seaweed Symposium）は，すでに本誌第36巻第1号でお知らせしましたように，カナダのブリティシュ・コロンビア大学で，1989年8月13日(日)～18日（金）に開催されることが確定しました。研究発表申込の締切は1989年2月15日（必着）です。

参加登録料は，2月15日までの申込については US $240（学生 140，同伴者 50），6月15日までの申込についてはUS $280（学生 160，同伴者 50），6月15日以後の申込については US $310（学生 240，同伴者 50）です。

研究発表申込および参加登録の用紙は，下記のシンポジウム事務局に申込んでください。本件に関するお問合せは，東京水産大学　有賀祐勝宛にお願いします。

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