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DOCTOR OF PHILOSOPHY

of

LOUIS ANTHONY HANIC

B.A., The University of British Columbia, 1950

THURSDAY, JANUARY 14th, 1965 AT 2:00 P.M.

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LIFE HISTORY STUDIES ON UROSPORA AND CODIOLUM FROM SOUTHERN BRITISH COLUMBIA

ABSTRACT

Life history studies were conducted on Urospora and Codiolum from a number of localities in the Strait of Georgia and Juan de Fuca Strait.

Urospora wormskioldii (Mertens) Rosenvinge (n = 12) is a dioecious species having a heteromorphic life history with filament, dwarf and Codiolum stages. Asexual reproduction is by means of acuminate quadriflagellate zoospores. The Codiolum stage is produced from zygotes, female and probably male gametes. The range in variability of morphological features of the vegetative filament is very great and encompasses all species now recognized from the Pacific Coast of North America. Because of this variability, the taxonomy of Urospora should be based on life history features as well as morphological ones. Matings were successfully made involving plants from six widely-separate localities. Sexuality occurred in cultures at 10°C, through several serial subcultures in the female but only in the first culture in the male. A method employing a long thermoperiod was successful in inducing sexuality in several vegetative clones of Urospora but failed in others. Temperature, filament size and nutrition appear to be factors involved in the sexual response. Fertility of cultural Codiolum was low and attempts to increase it met with only partial success.

Urospora vancouveriana (Tilden) Setchell and Gardner (n = 9) is an asexual species, producing a filamentous and dwarf stage via quadriflagellate zoospores at low temperatures and a Codiolum stage via biflagellate zoospores at high temperatures. Cultural filaments are indistinguishable from those of U. wormskioldii.

Urospora speciosa (Carm.) Leblond ex Hamel (n = ?) is recorded for the first time in North America and was discovered to be uninucleate. It contains a filamentous and dwarf stage which reproduce via quadriflagellate zoospores. Other features of its life history are unknown.

Codiolum gregarium A. Braun and C. pusillum (Lyngbye) Kjellman, in the areas studied, are considered to be merely form variants belonging to the life history of Urospora wormskioldii. Fertility in natural Codiolum

was inhibited by short daily warm thermoperiods and induced by cold treatment. Nuclear division was studied but meiosis was not demonstrated.

Codiolum petrocelidis Kuckuck, found as an endophyte in Petrocelis is inferred to belong to the life history of Spongomorpha coalita (Ruprecht) Collins in the areas investigated. It is considered to be unicellular, uninucleate and capable of reversing its direction of growth. The direction of growth is suggested to be governed by light intensity.

Cytochemical studies were done on the walls of Urospora, Spongomorpha and their Codiolum stages. The inner walls of Urospora and Spongomorpha are composed of cellulose and pectic materials, while those of the Codiolum types are entirely pectic. The pectic component appears to differ from that of higher plants. Urospora, Spongomorpha and their Codiolum stages have an outer sheath of unknown composition. Three types of sheaths are represented, of which those of the two Codiolum types are the same. To be of full taxonomic value, future wall studies should include all stages where algae with heteromorphic life histories are concerned.

Spongomorpha coalita was discovered to have operculate gametangia. This species should therefore be transferred to the genus Acrosiphonia. The implications of this transfer and the recognition of a unicellular condition for Codiolum petrocelidis are discussed in relation to life history and taxonomic problems existing in the Acrosiphonia-Spongomorpha complex.

GRADUATE STUDIES

Field of Study:

Genetics	K. M. Cole, H. J. Muller & R. P. Wagner
Cytogenetics	K. M. Cole
Cytology	R. E. Cleland
Phycology	R. F. Scagel, R. C. Starr & J. R. Stein

SCHOLARSHIPS

1960 - B. C. Sugar Refining Co. Ltd. Scholarship

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to the required standard

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Date January 17, 1965

ABSTRACT

Life history studies, emphasizing cultural and cytological approaches, were conducted on Urospora and Codiolum from a number of localities in the Strait of Georgia and Juan de Fuca Strait.

Urospora wormskioldii (Mertens) Rosenvinge (n = 12) is a dioecious species having a heteromorphic life cycle with a filamentous, dwarf and Codiolum stage. The Codiolum stage is produced by syngamy of anisogametes or by parthenogenetic development of female and probably male gametes. All three stages produce asexual quadriflagellate zoospores which develop into filament or dwarf plants. The range in variability of morphological features of the vegetative filament is very great and encompasses all species recorded from the Pacific Coast of North America. Crosses involving plants from six widely separate localities showed no incompatability at the mating level. Sexuality occurred spontaneously at 10°C in cultures of both sexes through several serial subcultures in the female but only in the first culture in the male. Temperature changes, filament size and nutrition appear to be factors involved in the sexual response. Several vegetative clones, including a two-year-old isolate from Cape Cod, were sexually induced by a method involving a long thermoperiod. However, the same method failed on other clones. Growth of Codiolum in culture was poor and it is suggested that daily exposure may be a requirement for normal growth. The fertility of zygotic and parthenogenetic Codiolum was low in cultures grown under constant temperature conditions. Attempts to induce fertility by cold treatments met with success in some isolates but not in others.

Urospora vancouveriana (Tilden) Setchell and Gardner (n = 9) is

an asexual species, producing filamentous and dwarf stages via quadriflagellate zoospores at low temperatures and a Codiolum stage via biflagellate zoospores at high temperatures. Cultural filaments are indistinguishable from U. wormskioldii.

Urospora speciosa (Carm.) Leblond ex Hamel (n = ?) is recorded for the first time in North America and was discovered to be uninucleate. It contains filamentous and dwarf stages which reproduce via quadriflagellate zoospores. Other features of its life history are unknown.

Codiolum gregarium A. Braun and C. pusillum (Lyngbye) Kjellman, in the areas studied, are considered to be merely form variants belonging to the life history of Urospora wormskioldii. The living nucleus and its staining characteristics are described. Nuclear divisions were followed but meiosis was not demonstrated. Fertility in both types was induced by cold and inhibited by short daily thermoperiods.

Codiolum petrocelidis Kuckuck, found as an endophyte in Petrocelis franciscana Setchell and Gardner is a unicellular, uninucleate plant producing ovate quadriflagellate zoospores which give rise to branching filaments. C. petrocelidis, as found in the areas studied, is inferred to belong to the life history of Spongomorpha coalita (Ruprecht) Collins. The Codiolum cell is capable of reversing its direction of growth and this reversal is suggested to be governed by light intensity. It is also proposed that the mode of stipe growth rules out a multicellular condition. Operculation was discovered in S. coalita.

Cytochemical studies were done on the walls of Urospora, Spongomorpha and their Codiolum stages. The inner walls of Urospora and Spongomorpha are composed of cellulose and pectic materials, while those

of the Codiolum types are entirely pectic. The pectic component appears different from that of higher plants. Urospora, Spongomorpha and their Codiolum stages have an outer sheath of unknown composition which is singly refractive, gives a negative test for cellulose, chitin, pectin and fat. Three types of sheaths are represented, of which those of the two Codiolum types are the same.

Several taxonomic implications result from these studies. To be meaningful, identification of Urospora species should be based on more than just purely vegetative features. To be of taxonomic value wall studies should include all stages where algae with heteromorphic life histories are concerned. Because of its operculate condition, Spongomorpha coalita should be transferred to the genus Acrosiphonia. This transfer would provide further evidence for the occurrence of a heteromorphic life history in Acrosiphonia. It would also re-establish Wille's basis for recognition of the two genera on nuclear condition. Acceptance of a unicellular condition for Codiolum petrocelidis, and a heteromorphic life history for Acrosiphonia would re-establish the basis for Jónsson's new family, the Acrosiphoniaciae and at the same time remove the main barriers to the inclusion of the Acrosiphonia-Spongomorpha complex in the Ulotrichales (sensu Kornmann).

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Life History studies on Urospora and Codiolum from southern British Columbia

Introduction

The genus Urospora, of the Cladophorales, was established by Areschoug in 1866. It comprises individuals which are filamentous, unbranched, having a rhizoidal holdfast formed by several basal cells. The vegetative cell has a netlike parietal chromatophore containing many pyrenoids, many nuclei and a large central vacuole. Asexual reproduction is by means of quadriflagellate posteriorly pointed zoospores and sexual reproduction by means of biflagellate iso- or heterogametes. About fifteen species are described inhabiting the cooler waters of the northern hemisphere. Setchell and Gardner (1920) recognized seven species from the west coast of North America of which six are recorded from British Columbia and northern Washington (Scagel, 1957).

The genus Codiolum, formerly contained in the Chlorococcales, was established by A. Braun (1855). It is comprised of small unicellular plants, both free-living and endophytic. The cell has a basal stalk, a parietal chromatophore with several to many pyrenoids, and one nucleus. Reproduction is by means of quadriflagellate zoospores. About nine species are described, of which all are marine except Codiolum lacustre Printz, which is freshwater. The three species described from the west coast of North America (Setchell and Gardner, 1920) are also recorded from British Columbia and northern Washington (Scagel, 1957).

In 1933 Jorde brought these two genera together in her report that Codiolum gregarium A. Braun (a free living form) belongs to the life history of Urospora mirabilis Areschoug and thereby demonstrated the

occurrence of a heteromorphic life history in the green algae for the first time. Though Jorde's results remain unconfirmed in relation to the life cycle of U. mirabilis, a similar Codiolum stage has been reported in other species of Urospora (Kornmann, 1961 b, c) and in other genera: Ulothrix (Kornmann, 1963, 1964a) Spongomorpha (Hollenberg, 1957, 1958; Fan 1957, 1959; Jónsson 1959b, 1962 and Kornmann 1961a); Acrosiphonia (Jónsson 1957, 1958, 1959a, 1962, 1963, 1964a, b); Monostroma and Gomontia (for pertinent literature see Kornmann, 1962, 1963, 1964b), and Cladophora (Archer and Burrows, 1960; Van de Hoek, 1964). However, these reports are not without conflict. According to Kornmann (1962), Acrosiphonia lacks a Codiolum stage and has an isomorphic life history, whereas Jónsson claims the opposite. Kornmann (1961a) believes C. petrocelidis Kuckuck and Chlorochytrum inclusum Kjellman to be merely endophytic modified forms of the zygote of S. lanosa, while Jónsson claims the former belongs to the life history A. spinescens (= A. arcta, Kornmann 1962) and the latter to S. lanosa. Most investigators e.g. Kuckuck (1894), Zimmerman (1925), Printz (1926), Hollenberg (1958), Fan (1959), Jónsson (1958, 1962) considered C. petrocelidis to be unicellular, while Kornmann (1961a) suggests it is multicellular. Van de Hoek (1964) regards the Codiolum-like cells in Cladophora as growth-suppressed germlings resulting from nutrient depletion.

As a result of the recent findings mentioned above, attempts at taxonomic revisions have been made. Den Hartog (1959) proposed the new family Codiolaceae to include Urospora species. However, with the discovery of other Codiolum-containing genera the basis for this family is removed. Jónsson (1959b) created the family Acrosiphoniaceae to include

Urospora, Spongomorpha and Acrosiphonia on the basis of having in common a unicellular Codiolum sporophyte, similar cell wall, chloroplast pyrenoid and vacuolar structure. Kornmann (1962) rejected the Acrosiphoniaceae and redefined the old order, Ulotrichales, to include Ulothrix, Urospora, Monostroma and Gomontia. He excluded Spongomorpha and Acrosiphonia from this order mainly because they lacked a quadriflagellate acuminate zoospore and had a different wall structure (Nicolai and Preston, 1952) and secondly because he felt Acrosiphonia had an isomorphic life cycle and Spongomorpha, a multicellular Codiolum zygote. As a result of these conflicting reports and different interpretations, the taxonomy of the marine green algae is, at present, in a fluid state.

Cytological studies on Codiolum-containing genera are restricted to those of Hart (1928) and Jorde (1933) on Urospora and Jónsson (1962) on Acrosiphonia and Spongomorpha and have been concerned mainly with the filamentous stage. Hart described the nuclear condition of germlings and filaments of U. wormskioldii. Jorde studied nuclear division in U. mirabilis and gave this species a chromosome number of four, but with some reservations since the chromosomes were very small. Jorde failed to observe division in the Codiolum stage. Jónsson (1962) reported that nuclear division in the filament of Spongomorpha and Acrosiphonia during gametogenesis is mitotic, thereby providing indirect evidence for meiosis in the Codiolum stage. However, Moewus (1938) demonstrated meiosis in the Codiolum stage of Monostroma more directly by obtaining a 1:1 sex ratio from the Codiolum products.

Our present knowledge of life histories in Urospora is confined to European types. Little is known about the genus from North America.

Setchell and Gardner (1920) attempted to arrange the species reported from this coast, relying largely on herbarium material. Several species were placed into synonomy by them. The species were separated on the basis of differences in rhizoidal structure, cell proportions and dimensions, chloroplast structure and size of fertile sporangia. These authors pointed out the need for studies on living material. Frye and Zeller (1915) described U. tetraciliata, from San Juan Island, from sexual material but did not follow zygote development. The unusual nature of gamete fusion, described as starting posteriorly, has been questioned by Collins (1918, p. 86) and Setchell and Gardner (1920, p. 195). Collins suggests that these may represent imperfectly separated microzoospores (= gametes). This species has not been recorded since, even though Frye attempted to relocate it at later dates (Setchell and Gardner, 1920). Hart's (1928) cytological studies were done on U. wormskioldii from San Juan Island and included a minor amount of culturing. She was the first to discover dwarf plants and thick walled resting cells in culture and to note their hardy qualities. Fan (1959) attempted a cultural study of U. penicilliformis from California but gave up after failure to obtain sexual material.

At this point a brief summary of the life histories thus reported in Urospora is warranted. The life history of Urospora mirabilis is shown in Fig. 1 and that of other Urospora species and Ulothrix, schematically in Fig. 2. U. mirabilis (Jorde, 1933) has three somatic stages: a filament, dwarf and Codiolum stage. It is dioecious and reproduces the filament and dwarf stage asexually via quadriflagellate zoospores. The Codiolum stage is produced from zygotes via sexual fusion of biflagellate anisogametes or from female parthenogametes. Meiosis is suggested to

occur in the diploid Codiolum cell

U. speciosa (Carm.) Leblond ex Hamel 1931, differs from U. mirabilis in being monoecious and isogamous (Kornmann, 1961c), (see Fig. 2, III).

U. penicilliformis (Roth) Aresch. 1866, is similar to U. mirabilis differing only in filament and gamete morphology (Kornmann, 1961c), (see Fig. 2, V).

U. wormskioldii (Mertens) Rosenvinge 1893, has not been found sexual in nature and therefore its life history is unknown

U. wormskioldii var. biflagellatum Kornmann 1961, lacks gametes. The filament and dwarf stage are produced from quadriflagellate zoospores and the Codiolum stage from biflagellate zoospores, (Kornmann, 1961 b, c), (See Fig. 2, VIa)

U. wormskioldii var. caudatum Kornmann 1961, produces a Codiolum phase via the quadriflagellate zoospores but lacks a sexual phase, (Kornmann, 1961 c), (See Fig. 2, VIb)

U. bangioides (Harv.) Holm. et Batt. 1890, has no sexual phase, or Codiolum stage, and reproduces only via quadriflagellate zoospores, (Kornmann, 1961 c), (See Fig. 2,I)

U. tetraciliata Frye and Zeller 1915, is reportedly isogamous but details on zygote development are lacking.

The present study was undertaken to obtain information on the distribution, morphology and life history of Urospora and Codiolum species from southern British Columbia. Endophytic Codiolum forms were also included since it could not be assumed that only free-living forms give rise to Urospora. Special emphasis was placed on cytological and cultural

approaches. The main objectives were to establish the alternation of generations on a cytological or genetical basis and to determine the conditions required to bring about the complete life cycle in culture. The latter involved studies on the sexual response in Urospora and the fertility response in Codiolum.

II. MATERIALS AND METHODS

Collections

Approximately thirty areas were investigated in the Strait of Georgia and Juan de Fuca Strait (see Fig. 3 for localities and Table 1 for their description). Three of these, Deadman's Bay (Fig. 4 G), Friday Harbor (Fig. 28 B) and Tsawwassen (Figs. 4 A and B) received monthly inspection for at least one year. Point No Point (officially known as Glacier Point)(Fig. 4 F), Ogden Point Breakwater (Fig. 4 C), Oak Bay (Fig. 4 D), Anacortes and Porlier Pass were visited two or more times during the summer and winter of at least one year. The remainder were visited infrequently. Nineteen of the areas (Fig. 3) were examined during a one week period in June, 1963, to obtain comparative data on the distribution, morphology and sexuality of Urospora. At each site samples of Urospora and Codiolum were taken throughout their vertical range and examined on site with a dissecting microscope at magnifications of 25, 50 and 100. Where Urospora or Codiolum were not visible, scrapings were taken from rocks and logs throughout the intertidal area with the aid of a wire brush and syringe. Species of Urospora and Codiolum collected and later identified were; U. wormskioldii, U. vancouveriana and U. speciosa, C. gregarium and C. pusillum. C. petrocelidis Kuckuck (obtained from Petrocelis franciscana Setchell and Gardner) and Spongomorpha coalita were collected from Deadman's Bay and Porlier Pass during the summer of 1961 and the summer and fall of 1963.

Culture techniques

Clones of Urospora were established representative of the upper, middle and lower range at each locality where a wide range occurred. The

morphology of the clones was compared with that of their field counterparts. Unless otherwise indicated cultures were grown and maintained in 20 x 180 mm test tubes containing 20 ml of UR1 medium (see below) at 10° ± 2°C, under 300 - 400 f.c. of illumination supplied by "cool-white" Sylvania fluorescent tubes on a 14 hr/10 hr light/dark cycle. Single germlings (6 - 12 celled) derived from mass zoospore cultures were used as the inoculum. The UR1 medium is equivalent to the SW1 medium of Iwasaki (1961) minus the TRIS, supplemented with 6 μ g/l of biotin, 10 μ g/l of thiamine and 0.02 μ g/l of vitamin B12. To minimize precipitation, the sterile medium was prepared by steaming one to two litre quantities of filtered seawater on three consecutive days, following which, the minerals and vitamins were added from three pre-sterilized stock solutions, one containing the nitrate and phosphate, one the FeEDTA and one, the vitamins. The stock solutions were sterilized by autoclaving for three minutes at 15 psi. A 1.5% agar preparation of UR1 was used to plate out zoospores and gametes. Other containers, as well as test tubes, used for experimental studies, were: 2 x 10 cm Petri plates (shallow) containing 50 ml of medium, 8 x 10 cm Petri plates (deep) containing 125 ml of medium, and 125 ml Erlenmeyer flasks containing 100 ml of medium. Other media used to study Urospora growth were: Erdschreiber's solution (Starr, 1956), Iwasaki's (1961) SW1 and SW11 media and modifications thereof. Constant temperature of 5°, 10°, 15° and 20° were used for behavioral studies of Urospora, Codiolum and their motile reproductive structures. Methyl cellulose was used to slow down gametes for critical observation.

The Urospora wormskioldii clone URX1 (derived from a large low tide form at Deadman's Bay) was used to study growth in different solutions (Table 2), different salinities ranging from 5 - 50 °/oo and

different light intensities ranging from 25 - 400 f.c. Clone URX1 had produced Codiolum cells in great abundance on two occasions separated by over a year, yet evidence of gametes was never found at those times or in dozens of other subcultures.

Infertile cells of Codiolum petrocelidis (Deadman's Bay) with the host, Petrocelis, were cultured in Erdschreiber's solution in shallow petri dishes at 10° ± 2°C and at various light intensities (25 - 400 f.c.). One lot was cultured in the liquid phase and one on moist filter paper supported at the surface of the medium with a glass triangle. The Petrocelis cultured in the latter manner underwent disintegration over a two week period leaving the Codiolum cells easily freed. This was done by passing moistened tissue several times through a narrow pipette. Freed cells were recultured in the liquid phase.

Stain techniques

Initial studies employed an acetocarmine technique in which the material was fixed in 3:1 (ethyl alcohol: glacial acetic acid), heated in glacial acetic acid at 60°C for 4 hrs, stained in acetocarmine (Darlington and LaCour, 1960) for 12 hrs at 60°C in a screw cap vial and differentiated by applying a drop of 4% ferric alum to the edge of a slide preparation and heating for a few seconds over an open flame. Though satisfactory as a nucleolar and chromosome stain, the chromatin in interphase nuclei of Urospora and Codiolum remained clear. Subsequently an intensive stain investigation was undertaken, which resulted in the adoption of three methods: a Newcomer-iron-propiocarmine method for Codiolum, a Feulgen-iron-propiocarmine method for Urospora and an aceto-orcein-iron-propiocarmine method for gametes. In the Newcomer-iron-propiocarmine technique, Codiolum was fixed in Newcomer's fixative (1953),

heated in glacial acetic acid for 4 hrs at 60°C, washed, mordanted with 2.5% ferric alum for 5 min, washed and stained on a slide with a drop of propiocarmine (prepared in the same manner as acetocarmine) and differentiated by brief heating (1 - 3 seconds) over an open flame. This method also worked well on Urospora and C. petrocelidis. In the Feulgen-iron-propiocarmine method, Urospora was cultured on slides, fixed in 3:1 for 12 hrs, stained with Feulgen (Darlington and LaCour, 1960), using 8 min hydrolysis, 1 hr staining and 10 min in the sulfite bleach. On removal from the bleach, the slide preparation was stained with a drop or two of propiocarmine, mordanted a light purple blue with iron from an iron file and differentiated with brief heating (1 - 3 sec) over an open flame. Over-mordanting and over-heating brought out nucleolar and cytoplasmic details. In the aceto-orcein-propiocarmine technique, gametes contained in a drop on a slide, were fixed over osmic acid vapours, stained with a drop of aceto-orcein, washed by drawing water under the coverslip, and then stained with a drop of propiocarmine mordanted lightly with iron from an iron file, and differentiated with brief heating over an open flame. Crystal violet was used as a flagellar stain. For staining germinating zoospores of Urospora, a 2% colchicine in seawater solution was applied for 9 hrs following the commencement of the dark cycle on the day when first cell divisions were observed (usually on the 4th day). Aniline blue in 10% HCl was used to stain C. petrocelidis in fresh or formalin fixed Petrocelis using mild heating to obtain separation of the Petrocelis filaments. For nuclear studies, C. petrocelidis was fixed in 3:1 and stained with acetocarmine. The stained material was then drawn through a narrow pipette to bring about further separation of the Codiolum from the Petrocelis tissue.

Wall Studies

Cytochemical tests were done on fresh material and material fixed in 3:1 or 5% formalin. Ruthenium red (diluted to 1 part in 5,000 with water) was used to test for the presence of pectic compounds, Sudan III for fats and IKI - H_2SO_4 or $ZnClI_2$ (both prepared according to McLung, 1937) for cellulose.

The presence of chitin was tested according to Jensen (1962) on unsectioned material. In this test the material was autoclaved in 23 M KOH in a screw-cap vial for 15 min at 15 psi and subsequently treated with IKI and 1% H_2SO_4. A violet-blue color indicated the presence of chitin. Schweitzer's reagent (Handbook of Chemistry and Physics, 1962 - 1963, Method 2, page 1650), HCl - $ZnCl_2$ (1 pt conc. HCl: 1 pt $ZnCl_2$ by weight) and slug cytase were used as cellulose solvents. The slug cytase was obtained from 3 - 6 in specimens of the slug Airiolimax columbianus Gould, starved for 12 hrs. The cytase was preserved with a few crystals of phenol according to Fabergé (1945) and used undiluted immediately after extraction. Except where heat or long periods were involved reactions were observed in progress at magnifications of 100 or 450 times.

Urospora matings

Field material to be used for mating was stored at 10°C, the temperature at which gamete formation was most prolonged. Mating of Urospora wormskioldii gametes was done by cooling filaments of both sexes on ice in the dark for about 30 min, transferring one or two filaments of each sex to a small drop (5 - 7 mm diameter) on an ice-cooled slide, and illuminating with a bright light to stimulate gamete release. The slide was transferred to a cooled microscope stage and copulation was observed at magnifications of 100 or 450 times. The slide preparation

was fixed over osmic acid vapours, stained with propiocarmine - lightly mordanted with iron from a file, and made permanent in Euparal. Dehydration and embedding were done in a chamber saturated with ethyl alcohol. Several crosses were made of 8L female (Long Beach) with 18 - 3 male (Tsawwassen) for cultural purposes. In this case a dense male gamete suspension was prepared in a small drop about 0.5 mm diameter, and into this 50 to 100 female gametes were inserted.

Induction of sexuality in Urospora and fertility in Codiolum

The experiments on induction of sexuality in Urospora wormskioldii were done mainly with female clone F11, male clone M2A (both from Tsawwassen), clone URX1 (Deadman's Bay), and secondarily with asexual isolates derived from Codiolum or unknown Urospora types and with a two-year-old Urospora clone from Cape Cod, Massachusettes. The experiments on the induction of fertility in Codiolum were done with cultural Codiolum from clones F11, M2A, URX1 (localities given above), female F8 (Long Beach), F3 female (Oak Bay), and natural Codiolum from Deadman's Bay, Friday Harbor, Tsawwassen, Oak Bay, Point No Point, and Ogden Point Breakwater. The methods used are given together with the results.

Tide level determination

The tidal levels given are approximate and are based on interpolations from tide table data (Anon, 1962 - 3) with respect to measurements of water levels taken at specific times. Levels at Deadman's Bay and Friday Harbor were determined from the tide staff on the cantilever pier at the Friday Harbor Marine Laboratories, taking the zero mark and tidal amplitude as being roughly equal to that given for Fulford Harbour, B. C.

III. RESULTS AND OBSERVATIONS

1. Type I. *Urospora wormskioldii* (Mertens) Rosenvinge

Ecology (Life cycle, Fig. 7)

Urospora wormskioldii was the most common species encountered in the areas studied. It is a mid-tide form ranging from the mean of the higher high waters down to the mean of the lower low waters (Fig. 5). Occasionally it is found during the winter in sparse quantities at high tide associated with fertile cells of *Codiolum gregarium* A. Braun and in the spring, (March to early June) in abundant quantities in the low tide. In the spring and early summer *U. wormskioldii* is associated in the mid-tide with *Porphyra lanceolata* (Setchell and Hus) G. M. Smith, *Bangia fuscopurpurea* (Dillwyn)Lyngbye and *Ulothrix* species, mainly *Ulothrix flacca* (Dillwyn) Thuret. In its smallest form (high tide) *U. wormskioldii* resembles *U. penicilliformis* (Roth) Areschoug and in its largest form (low tide), *U. vancouveriana* (Tilden) Setchell and Gardner. In its upper range it frequently suffers from fungal infection and epiphytism.

Sexual plants occur from early April to late September and are entirely restricted to the mid-tide. At Tsawwassen plants become sexual on the south side of the jetty about a month sooner than those on the north side. *U. wormskioldii* becomes more scarce and appears lower in the intertidal as summer progresses and may disappear during June, July, and August in southerly exposures, i.e. southeast side of Tsawwassen jetty.

In most areas the coverage of *Urospora* is very spotty, often confined to patches on widely separate rocks. A continuous coverage is found mainly on uniform substrates, i.e. the flat surfaces of the granite blocks forming the Ogden Point Breakwater (Fig. 4 C), very large flat surfaced boulders, beaches composed of small smooth rocks four to twelve inches in diameter as occur at Tsawwassen (Fig. 4 A and B) or Oak Bay

(Fig. 4 D), debarked sunken logs, and pilings. On one occasion it was found on a newly painted Salmon Troller at the bow near the waterline and in one instance (Departure Bay), on a tractor tire in the mid-tide. However, it was never found as an epiphyte or in tide pools.

Very large asexual forms (Fig. 23) were found in the low tide region (0 - 2 ft) in the same place in the spring and early summer (April - June) of two succeeding years at Tsawwassen, Deadman's Bay, Point No Point, and once at Kelsey Bay. Except at Kelsey Bay these were associated above with sexual plants of U. wormskioldii. At Tsawwassen and Deadman's Bay the two types were distinctly separated, but at Point No Point they formed a continuous vertical band, the size of the filaments gradually increasing with depth. Attempts were made to induce sexuality in several clones of large low tide forms but these failed. As a result, their assignment to U. wormskioldii cannot be certain.

Urospora wormskioldii is remarkably resistant to desiccation and heat. After prolonged exposure of up to six hours in the hot sun the filaments can be rubbed to a powder between the fingers. Yet the plants swell up quickly on rewetting and, if fertile, release masses of zoospores. The same behavior was observed for cultural plants. After five days of drying at 20° - 23°C for four hours daily, active zoospores were released on rewetting. Field plants can also withstand periodic heavy rains and freezing conditions. Dwarf plants survive cold much better than the filaments. When field filaments and cultural dwarf plants were kept in the dark in frozen seawater (-5°C) the filaments died within three days, whereas the dwarf plants survived very well for more than three months. Also, dwarf plants survived at salinity extremes of 10 °/oo and 50 °/oo for over a year. However, in spite of these hardy features of Urospora,

its abundant spore production and its short life cycle (10 - 20 days). Urospora is not as abundant or widespread as might be expected.

Morphology of field plants

Vegetative plants vary considerably in dimensions and morphology from season to season or from locality to locality. The plant length varies from 0.5 - 24 cm and its width from 80 - 1200 μ. The holdfast is composed of 5 - 23 rhizoids, which generally are intramatrical in large plants (Fig. 10 A) but occasionally extramatrical in small ones. The number of rhizoids increases with the size of the plant. Usually, basal cells are shorter than wide (Fig. 10 A), subcentral cells isodiametric and slightly swollen, and more distal cells barrel shaped or spherical (Fig. 10 D). Generally the cylindrical cell is a feature of small plants and the barrel or spherical cell of large plants.

The chloroplast in small cells is parietal, in large cells cylindrical, and occasionally is found withdrawn from the end walls in elongated basal cells (Fig. 8 A). The chloroplast varies from being coarsely reticulate in basal cells (Fig. 8 B), especially elongated ones, to finely reticulate in larger distal cells (Fig. 8 G and 10 B, E). Coarse reticulation, however, may occur in larger cells, especially at the end walls. This feature seems to be characteristic of rapidly growing filaments, for in culture the perforations become smaller with age of the filament.

Occasionally cells may be found in which the chloroplast consists of unconnected bands (Fig. 8 F, upper cell) arranged in a helical fashion. These are most often found next to dead cells or in cells growing down through dead cells (Fig. 10 A). The latter type of cell was observed in stained material as well. In all cases the nuclei of the cell

being perforated were degenerate. In several instances the nuclei in the cell adjacent to the one being perforated were also degenerate indicating that the cells were dying.

Sexual plants, on the other hand, are much more uniform, varying from 100 - 300 /u in width and 2 - 4 cm in length. The vegetative cells and gametangia are most often isodiametric, or shorter than wide, and slightly swollen. All but the rhizoidal cells may become fertile to produce quadriflagellate zoospores, or sexual to produce biflagellate gametes. These are liberated through a pore (Figs. 12, D and G) formed by gelatinization of the inner walls (Figs. 9 A, B and 27 G). On liberation the outer membrane is ruptured and the spores are released in a vesicle (Figs. 9 D and E) or stream; the female gametes in a stream and the male gametes usually in a vesicle. Gametangia and small sporangia have a single discharge pore centrally located, but larger sporangia may have two to four pores located near the end walls.

The zoospores (Fig. 10 H) range in size from 15 - 20 /u in length and 5 - 7 /u in width and are usually arranged parallel to the surface of the cell (Fig. 10 F). In large cells they may be arranged in star-shaped clusters. The posterior tip of the zoospore may be long and thin (Fig. 10 H) or short and gradually tapered. In cross section the zoospores are quadrate (Fig. 27 K), but later round up (Fig. 27 L). The zoospore chloroplast is parietal and has four anterior projections (Fig. 25 B, 27 K and L). Four fibril-like structures (Figs. 11 E and 27 K, J) originate between the points of flagellar insertion and pass backwards along the four ridges of the wall. The flagella are stiff and gradually taper to the end. An area at their base appears very sensitive to external conditions, as in slide preparations, the flagellar sheath at

this point swells into a balloon-like structure (Fig. 21 D) before the flagella are cast off. Detached flagella may exhibit flicking motions for several minutes and therefore show a degree of autonomy.

Male and female gametangia are formed on separate plants with zoosporangia (Fig. 11 D). Similar to those in culture, they are usually isodiametric (Fig. 11 D) but may be longer than wide (Fig. 13 A and F). Female gametangia are of the same color as zoosporangia, a dark green, while male gametangia are yellow-orange. Unlike the vacuole of the zoosporangium, that of the male and female gametangium frequently contains several discrete semitransparent globular masses 10 - 20 /u in diameter.

Early-formed male gametes are spindle-shaped (Figs. 12 L and 13 K), and average 8 /u in length and 2.5 /u in width. Their spindle shape is readily detectable in gametangia with newly formed gametes. (Fig. 13 F and G). On aging, the male gametes become ovate or spherical (Fig. 11 D) and in this condition do not fuse with female gametes. The chloroplast is cupshaped and lacks an eyespot. Early formed female gametes are ovate (Fig. 13 A), but become quite asymmetrical within a few hours. The body develops two or three ridges and after becoming slightly curved and twisted (Fig. 12 K and 13 B, C) averages 4 /u in width and 14 /u in length. The chloroplast is cupshaped and contains a prominent posteriorly located eyespot. Both male and female gametes have two flagella, averaging 20 /u in length, which become abruptly thinner at the tip. Occasionally both were found with four flagella of equal (Fig. 13 I) or unequal size (Fig. 13 H). Both have a posteriorly located pyrenoid which in the male is only visible on staining (Fig. 13 J). Male gametes move rapidly through the water in more or less straight lines while the females move along a shallow spiral path. The anterior region of female gametes is very

metabolic in that it may stretch, shorten, twist, or rotate very much like Euglena. This feature is readily observable in gametes left behind in a newly discharged gametangium. Neither male nor female gametes showed any phototactic response over intervals of an hour when subjected to light intensities ranging from one to a thousand foot-candles. However, gametes produced in culture, invariably settled in a very narrow band (0.1 mm) at the air/water/glass interface whether the cultures were illuminated from above or below. With side illumination the gametes generally settled at the interface with greatest density nearest the light source.

Gamete fusion is very rapid under ideal conditions, taking one to two minutes from the time of contact. Pairs fasten to each other by their anterior ends (Fig. 14 A upper left) and rotate to a lateral position during fusion (Fig. 14 A lower row). The quadriflagellate zygotes remain active for some time (at least an hour) but show no phototactic response. Occasionally two females were observed copulating with one male (Fig. 14 A middle right). Male gametes are much more sensitive than females to external conditions. On warming the slide, the males quickly round up and attach to the slide, while the females may exhibit normal motion for fifteen to twenty minutes longer. Nine successful matings were made between male and female gametes involving plants from six widely separate localities (Figs. 3 and 23). Zygote development was followed only for the cross between female 8L (Long Beach) and male 18 - 3 (Tsawwassen).

Culture

Zoospores from field plants remain active at 10°C up to four days and, after attaching to the substrate, lose their flagella, germinate

to form either filaments (Figs. 11 A - D and 12), dwarf plants (Fig. 9 F), or both, depending on the culture conditions. Filament growth, though initially good in filtered seawater alone, declined through serial subculturing. Addition of nitrates and phosphates failed to improve growth. Of other media tested, growth was much better in Erdschreiber's solution but was inconsistent from culture to culture. Best and most consistent growth was obtained in Iwasaki's (1961) SW1 and SW11 media (Table 2).

The degree of zoospore germination varied and appears to depend on the concentration of zoospores used, the quality of the seawater, and the quality of the spore itself. In initial trials, 180 single zoospores, obtained from 18 plants (Tsawwassen, 1961) failed to germinate in Erdschreiber's solution. Similarly, zoospores from the same plants in sparse concentration or cultured singly failed to germinate in drop cultures down to 3 mm in diameter. On the other hand, germination was good when high concentrations of zoospores were used regardless of drop size. Curiously, single germlings of only a few cells (4 - 9) originating from drop cultures with high zoospore concentration, grew very well in seawater alone, indicating that the zoospores have a critical germination period. At a later date, however, it was found that single zoospores derived from a female filament F11 obtained from Tsawwassen (1963) germinated in seawater alone but growth was much retarded (Table 3). Subsequently it was found that normal growth in the female cultures (Table 3) could be restored by addition of a small amount of SW1 medium which contained a supplement of nitrate, phosphate, and iron. On the other hand single zoospores from cultural filaments of male clone M2A (Tsawwassen) failed to germinate in seawater alone. However, these did germinate in media supplemented with FeEDTA (Table 4). Zoospores from other cultures

and from fertile Codiolum cells behaved in the same way, indicating that this critical germination period is a common characteristic of Urospora and Codiolum zoospores.

Cultural filaments show little correlation to their field counterparts and lack distinction between isolates. Under crowded conditions they vary from 2 - 4 cm in length and 100 - 200 /u in width and, in most cases, taper toward the base and apex (Figs. 11 A and 12). In uncrowded cultures the filaments may exceed 30 cm in length in which case they usually form rope-like strands. However, these long filaments are seldom wider than 200 /u. The rhizoids which number from 5 - 15 are, unlike their field counterparts, always extramatrical (Figs. 11 A and 12 A) and frequently ramified at their tips (Figs. 12 I and J). Rhizoids may also be formed at sharp bends in the filament as in Urospora vancouveriana (Fig. 17 F) or at the tip when it reaches the air/water interface. Basal cells are generally shorter than wide, cylindrical or slightly swollen; central cells are mostly isodiametric and often swollen, while apical cells are frequently very elongate (Fig. 12 N). Cell proportions, however, vary from culture to culture of the same clone and in some cases the cells, throughout the filament length, may be up to ten times as long as wide. The chloroplast in cells of young rapidly growing filaments is usually coarsely reticulate and becomes finely reticulate in larger or older cells. Chloroplasts retracted from the end walls were never found in culture and therefore appear to be an environmental feature. Cultural zoospores show the same variability in shape as field types but are generally smaller, averaging 10 /u in length and 4 /u in width. However, in very large sportangia, the zoospores may reach 18 /u in length. Cultural sporangia as well as gametangia have a single pore. Both male and female gametangia in cultural

filaments are yellow-orange. The size, morphology and behavior of the gametes are the same as those from the field.

Clones from all localities produced dwarf plants (Fig. 9 F) as well as filaments. The former consist of cell clusters attached to the substrate by their own cells and/or by rhizoids developing out of several basal cells. Dwarf plants can be recognized at a very early stage in development by the random planes of cell division. The cells have a central vacuole and a finely-reticulate parietal chloroplast with several pyrenoids in small cells or many in large ones. The proportion of dwarf plants and filaments depends on the cultural conditions. Dwarf plants increase with higher or lower salinities and are predominant at 10 °/oo and 50 °/oo whereas filaments predominate at salinities from 20 - 30 °/oo. Over the range of light intensities used (25 - 400 f.c.) dwarf plants decreased and filaments increased with an increase in light intensity. Dwarf plants have many rhizoids (Fig. 9 F) at low salinities (10 °/oo) and none (Fig. 9 H and I) at high salinities e.g. 50 °/oo. At these salinities they frequently produce large thick-walled cells up to 200 μ in diameter which have persisted in unchanged media for over a year. Cells of this type frequently have no vacuole and look very much like stipeless Codiolum cells. These are very easily freed from the plant and on transfer to fresh medium of normal salinity, produce typical quadriflagellate zoospores which give rise to either dwarf plants or filaments. Dwarf plants were occasionally encountered during the winter months in the field but never contained such large cells.

Male clone M2A and female clone F11 grew slowly at 5°C, best at 10° and 15°C, and only F11 grew at 20°C. It was not determined if other male clones failed to grow at 20°C.

Of ten male and twenty-four female cultures grown at 10°C, one male (M2A) and two female (F11) cultures were found producing gametes after four months. Five of the males and fifteen of the females (including the sexual clones) contained typical Codiolum cells (Fig. 14 B and C) which were most concentrated in a thin ring, less than 1 mm wide at the air/water/glass interface (Fig. 8 E) and densest nearest the light. Codiolum cells derived from male and female cultures are termed male and female Codiolum respectively. Subsequently 10 clones of male (M2A) and 15 of female (F11) were serially subcultured twice. None of the male clones became sexual or produced Codiolum cells; whereas most of the females did (Table 3). Over further subcultures sexuality was also lost in the female. Of the five male clones which produced Codiolum, three originated from Tsawwassen, one from Oak Bay and one from Long Beach.

The origin of Codiolum in male cultures could not be determined. When cultured in liquid or agar media at various temperatures, zoospores from field and cultural plants gave rise to Urospora but never Codiolum and male gametes always died. Furthermore, though male M2A was made sexual later on a number of occasions, the cultures never produced Codiolum.

Female gametes and zygotes developed into Codiolum gregarium-like plants of about one-third to one-fifth the size of C. gregarium in nature. These have a parietal, seldom-reticulate, chloroplast containing several pyrenoids in small cells and many in large cells. The stipe of cultural plants is frequently much reduced and the layers within are quite uniform (Fig. 37 A). Only one zygote was found fertile after 24 days of culture and this produced normal quadriflagellate zoospores, which germinated in

the cell to form Urospora plants. Fertile haploid Codiolum cells from female clone F11 and F3 in all instances contained abnormal products: irregular-sized masses without flagella; zoospores with 2, 4, or up to 6 flagella; and in one cell two small Codiolum cells and a rounded up structure with an eyespot (Fig. 29 A). The latter cell was observed in cold shocked material. (Details p. 31). In liquid and agar cultures the zoospores germinated within the Codiolum cells, the cell wall gelatinized and filaments or dwarf plants developed from the mass of germinating spores.

Cytology

The cells of filaments and dwarf plants of U. wormskioldii are multinucleate and nuclear division is synchronous. In the cells of rapidly growing filaments a concentration of nuclei is generally seen at either end wall and, occasionally, in longer cells in the mid-region as well (as in Fig. 19 G). Cell division is accomplished by a furrow (Figs. 33 D, F and as in Fig. 19 I) developing in the middle of the cell protoplast, gradually extending inward to divide the cell into two equal halves. Cell division is independent of nuclear division. Additional wall material is formed about the cell which can be distinguished from the outer common daughter layers. The common layers appear to undergo some reorganization in the area of cell division, for in other non-dividing cells the layers appear continuous about each cell with only the outer filament sheath being common (Figs. 27 G and 33 C). This reorganization, however, may not be complete since the cellulose layers around adjacent cells appear connected or incomplete when viewed after autoclaving in 23 M KOH (Fig. 34 A and C). When filaments from nature are allowed to stand in culture for several days, individual cells can be removed readily from the filament sheath

with their inner walls intact. This can be done simply by squeezing the cells out of the sheath with a dissecting needle. This would indicate that the connection between cells is not strong. One of these cells is shown in Fig. 11 F. An additional layer with an inner ragged surface was frequently observed within empty sporangia and appears to be formed during zoospore formation or soon after (Fig. 27 G). This layer may be instrumental in building up turgor pressure in the cell by reducing its inner volume. Occasionally the cell division furrow starts obliquely, resulting in the two cells being coiled about each other (Fig. 8 D).

In dwarf plants, the nuclei are frequently associated in random clusters. The large Codiolum-like cells of dwarf plants were very difficult to stain and were found to be multinucleate. Prior to spore formation in filaments, the nuclei divide rapidly, often remaining in clusters (Fig. 21 A). During this process the pyrenoids become very indistinct (Fig. 8 H) and the chloroplast granular. Initials of zoospores are first seen as spheres compressed at points of contact with other cells (Fig. 8 I) and later these elongate and develop flagella. Fertile cells which earlier had a coarsely-reticulate chloroplast (Fig. 11 G) reflect this reticulation in the arrangement of zoospores (Fig. 11 H). Nuclear clusters are characteristic of large cells and are reflected in the star-like arrangements of zoospores mentioned earlier. Zoospores (Fig. 27 I) and gametes (Figs. 12 K - M and 13 D, J) have a single nucleus located anteriorly, but occasionally both were found with two nuclei. However, nuclear number is not correllated with flagellar number for zoospores and gametes with one or two nuclei had either the normal or double number of flagella.

The nuclei of filaments, dwarf plants, zoospores and gametes

have a prominent often vacuolated nucleolus with about 2 - 4 small Feulgen-positive chromatic bodies (previously undescribed) in close contact with it (Figs. 25 C, E and as in Fig. 20 A). The nuclei increase in size from about 4 /u in small germlings to 10 /u in larger filaments. With increase in nuclear size the number of chromatic bodies increases up to ten and these become more distant from the nucleolus (as in Figs. 20 G and I). The chromatic bodies probably represent heterochromatic portions of inter-phase chromosomes. Nuclei of germlings and zoospores stained with Feulgen, but larger nuclei of mature filaments did not. A good nuclear stain was obtained when the Feulgen method was followed by staining in iron-propiocarmine. The nuclear membrane and nucleolus disappear prior to metaphase. The chromosomes become oriented at the metaphase plate with their long axis parallel to the spindle apparatus. The chromosomes are seldom more than 2 /u long and 1 /u wide and appear most frequently as small round dots. (Figs. 26 L - N, Q and R). The chromosome number of U. wormskioldii based on counts from metaphases in young germlings is twelve.

Cultural Codiolum cells (zygotic and parthenogenetic) contain a single nucleus which, except in the very smallest young cells, fails to stain either in Feulgen or Feulgen-iron-propiocarmine. Since sectioned material behaves the same way, this lack of staining appears to be a property of the nucleus itself. The nucleus, however, stains well in Newcomer-iron-propiocarmine revealing the same type of vacuolated nucleolus and chromatic bodies as in Urospora. Nuclear division was not observed in cultural Codiolum.

In the living state the pyrenoid of Urospora (Fig. 10 E) and Codiolum appears to consist of a central spherical hyaline mass surrounded by several starch plates. When stained with acetocarmine, Newcomer-iron-

propiocarmine or Feulgen-iron-propiocarmine the central mass appears homogenous (as in U. wormskioldii, Fig. 20 A), but occasionally contains several small dark staining bodies in the centre. When the third stain is used on sectioned material the central mass appears to be compound (Fig. 21 E and F). However this may be an artifact of sectioning.

Wall cytochemistry (see Figs. 31, 33, 34)

The individual cells of Urospora wormskioldii are surrounded by a thick wall composed of several layers. All the cells are bounded on the outside by a thin filament sheath. The layers and sheath are easily distinguished when treated with IKI - H_2SO_4 (Fig. 33 E) due to the resultant swelling in the acid. The inner layers give a positive test for cellulose with IKI - H_2SO_4 or $ZnClI_2$ before and after heating in concentrated HCl, after autoclaving in 23 M KOH and after treatment with Schweitzer's reagent, or slug cytase. They are also doubly refractive under polarized light before (Figs. 33 A and B) and after (Fig. 33 G) boiling in concentrated HCl. The inner layers give a positive test with ruthenium red before but not after boiling in concentrated HCl indicating the presence of acid soluble pectic compounds. The wall material left after acid treatment is readily soluble in Schweitzer's reagent or slug cytase. The sheath gives a negative test for cellulose, pectin, fat, or chitin; is insoluble in slug cytase, Schweitzer's reagent, and is singly refractive (Fig. 34 F). It is soluble in HCl - $ZnCl_2$ (boiling) or 23 M KOH and in $ZnClI_2$, Schweitzer's reagent and IKI - H_2SO_4 after brief heating in concentrated HCl.

In the Codiolum cell, the protoplast is surrounded by layers of material which only give a test for pectic compounds and which are soluble in hot concentrated HCl. The whole cell is bounded by a thin

membrane different to that of Urospora but also of unknown composition. Other details on the walls are given under C. gregarium (P. 43) which gives the same tests and reactions as cultural Codiolum.

Induction of sexuality

Temperature

After the loss of sexuality in the male clones and its reduction in female clones when subcultured at 10°C, cultures of male M2A, female F11 and URX1 were cultured at 5°, 10°, 15°, and 20°C. Gamete production occurred in both sexes at 15°C and in the female at 20°C (Table 9) being highest in the female at 20°C, with about 50% of the filaments becoming sexual. However, on subsequent cultures of both sexes at 15° the percentage of sexual filaments dropped to less than 1%. During this period 500 test tube Urospora clones were established from 50 fertile Codiolum cells obtained from several areas at Tsawwassen and were cultured at 15°C, but in no case was sexuality evidenced over a two-month observation period. The medium in the cultures was then replaced and the cultures were divided into two lots, one being placed at 15°C, the other at 10°C. Again no sexuality was observed. These results clearly indicated that the sexual response can be restored or increased by culturing at warmer temperatures but not induced.

Thermoperiod and desiccation

Cultures of male M2A, female F11 and URX1 were grown in shallow Petri dishes at 10°C and after 10 days, when the filaments were 2 - 4 cm long and at their height of growth, one duplicate set received drying at 10°C and one at 20 - 23°C for 4 hrs on five consecutive days during the middle of the 14 hour daily photoperiod. After each treatment fresh medium was added and the cultures were examined for evidence of sexuality.

No sexuality was observed over the five day period in any culture. Death of cells and whole filaments occurred in all cultures, and was higher in those receiving a thermoperiod and highest in the male cultures. After this period about 50% of the male filaments and 10% of the female filaments were dead. Under these conditions dwarf plants, small filamentous germlings and the rhizoids and basal cells of large filaments survived best. The addition of fresh media each day resulted in fertile cells discharging zoospores which became active immediately on release. Three days following return to normal conditions (10°C) the two male and two female cultures which received a daily thermoperiod produced gametes in abundance. Gametangia occurred only in large filaments and comprised about 30% of surviving cells. Under these conditions periods of desiccation and heat, but not desiccation alone, increased sexuality in the female and induced it in the male. The effect of thermoperiod alone was then investigated.

Thermoperiod

Male (M2A), female (F11) and URX1 clones were grown in test tubes at 10°C for 10 days and then duplicates were transferred to thermoperiod boxes (Fig. 30) where they received daily warming for four hours during the middle of a 14-hr photoperiod on 15 consecutive days. The heat in each box was provided by wire wound resistors with various outputs and was controlled by an air thermostat with 0.1°C sensitivity. Temperatures were measured in, and at the bottom of the test tubes. After the 4-hr thermoperiod cold air (5°C), forced into the boxes by means of a pump, was used to bring the cultures down to the basal temperature. The lag in reaching the maximum temperatures and the variation of temperature between tubes (± 2°C) makes precise interpretations difficult. Nevertheless

the results have some value. The cultures at 4°C died in three days; those at 32°C, in 14 days, while the rest survived. The male filaments showed considerable bubbling of the sheath at 21°, 24°, and 29.5°C. On the fifteenth day a few gametes were observed in one of the two control female cultures and in one female at 29.5°C. A few Codiolum cells were observed in the female cultures at 10° to 32°C on the tenth day, which at the latter temperature were dead by the 14th day. On the 16th day the solutions were replenished and the cultures were returned to 10°C but no sexuality was evident over a three-week observation period. Under these conditions, thermoperiods were not effective in increasing or inducing sexuality; nor was a nutrient change following fifteen cycles of thermoperiods. Since a thermoperiod failed to bring about sexuality here, but did in cultures subjected to daily thermoperiods and nutrient change, it is suggested that nutritional factors may be involved as well.

Prolonged thermoperiod and nutrient repletion

Because of the high rate of death in cultures subjected to thermoperiod and exposures combined, and poorer growth of filaments in test tubes, it was decided to see if a long thermoperiod placed during maximum plant growth would induce sexuality. Male (M2A), female (F11) and URX1 clones were cultured in deep Petri dishes at 10°C for ten days and then were placed at 20°C for six days. After this time the filaments of all clones showed yellowing and vacuolation. The nutrients of one lot were replenished and the cultures returned to 10°C. After two to three days gametes were being produced in abundance in M2A and F11, but not in URX1, in which the nutrients were changed. Gametangia were again confined to larger plants. Subsequently this same method was successful in inducing sexuality in two clones from Point No Point and one from Cape Cod,

Massachusettes but failed on one clone of Urospora speciosa. The clone from Cape Cod had been maintained in culture asexually for two years previous indicating that temperature is a prime factor involved in the sexual response. It is quite possible that the success of this method was in part due to the larger size of the containers used, since filament production was much more abundant in these.

Induction of fertility in cultural Codiolum

The effect of temperature was studied on the growth of Codiolum resulting from female gametes of clone F3; on Codiolum plants from male clone M2A, female clone F11(both two months old), URX1 (four months old) and one-day-old zygotes (8L♀ X18-3♂). The F3♀ gametes were cultured on agar, the M2A, F11, URX1 Codiolum in shallow Petri plates and the zygotes on slides in Erlenmeyer flasks. The temperatures used were 5°, 10°, 15°C throughout and also 20°C for F3♀ gametes.

Only one zygotic Codiolum cell (8L♀ X18-3♂) became fertile over a two-month culture period, this being on the 24th day at 10°C. This one cell produced normal quadriflagellate spores which germinated within the cell. About 3% of the F3♀ Codiolum cells became fertile at 10° and 15°C and none at 5° and 20°C. All of these contained abnormal products, masses of varied size without or with many flagella and undivided zoospores in addition to normal spores. None of the No. 8L♀, M2A♂, F11♀ or URX1 Codiolum cells became fertile. In all cases the growth was retarded at 5° and 20°C and was best at 10° and 15°C. A heavy bacterial growth occurred in agar cultures at 15° and 20°C and in such cultures Codiolum cells were very small lacking a stipe.

Zygotes of (8L♀ X18-3♂) and Codiolum from F11♀ were subjected to a daily 8-hr thermoperiod of 18 ± 2°C (coincident with an 8-hr light

period) for two weeks and then were returned to 10°C. None became fertile. Plants of the same type were given a 4-hr drying period at 22 ± 2°C for one week and again none became fertile on return to 10°C.

Codiolum cells from No. 8L and URX1 were subjected to cold shocks of one or six day duration at -2°C under dark conditions. On return to 10°C only the 8L Codiolum cells became fertile (Table 7). Those cold-shocked for one day started to become fertile very much sooner, after three days, suggesting that longer periods of cold hasten fertility. In all cases these cells produced abnormal structures (Fig. 29). Unfortunately it was not possible to try this method on zygotic material.

2. Type II Urospora vancouveriana (Tilden) Setchell and Gardner

(Life cycle, Fig. 15)

Urospora vancouveriana * was found at Point No Point (Fig. 4 F) during July and August, 1963, where it formed a dense covering on rocks in sandy areas in the low tide region (0 - 2 ft). The plants were largest at the lowest level, here frequently exceeding 30 cm in length and 2 mm in width, and decreased in size upwards, where they merged with a band of sexual U. wormskioldii. The level at which one ended and the other began could not be established without resorting to extensive culturing of samples from the suspected transition zone. However, it appeared that this zone was between the two and three foot level. The following discussion of the U. vancouveriana is based on culture studies of six clones established from large filaments taken from six areas along a hundred foot horizontal stretch of the beach between the zero and two foot tide level.

The filaments of Urospora vancouveriana have a holdfast composed of 11 - 30 intramatrical rhizoids (Fig. 17 B), and taper gradually from just above the holdfast to the end of the filament (Fig. 17 A). Individual cells at the base are isodiametric and slightly swollen, becoming more swollen sub-centrally and finally spherical in more distal regions (Fig. 17 A). In the largest filaments the cells may reach 3 mm in diameter. The chloroplast is frequently coarsely reticulate in basal cells and finely reticulate in mature cells (Fig. 17 C). The zoospores, which average 18 μ in length, 7 μ in width, are arranged mostly parallel to the cell surface (Fig. 17 D) and are liberated through a central pore.

* This form was brought to my attention by Dr. M. Dube of Western Washington State College

Culture

The six clones were cultured at different temperatures (5°, 10°, 15°, and 20°C) and light intensities (25 - 400 f.c.) As in U. wormskioldii zoospores developed into filaments (Figs. 17, F - I) and dwarf plants (Fig. 17 J). Cultural filaments are indistinguishable from those of U. wormskioldii. The chloroplasts in the cells in rapidly growing plants are, as in U. wormskioldii, coarsely reticulate (Fig. 17 G) and become finely reticulate with age. The dwarf plants contained one or two types of sporangia: one type producing quadriflagellate zoospores (Fig. 18 B) and the other type, biflagellate zoospores (Figs. 17 J and 18 A, C). The latter type were readily distinguished by their yellow-orange color. Filaments produced quadriflagellate zoospores at all temperatures. Cultural zoospores are generally about two-thirds the size of natural ones (Fig. 17 E). The number of dwarf plants increased with a decrease in light intensity and increase in temperature, while the reverse was true of filaments. Yellow orange sporangia increased with an increase in temperature (Fig. 16) and decreased with a decrease in light intensity.

The biflagellate zoospores are released in vesicles (Figs. 18 A, D, E and 19 F) and this release can be induced by cooling the culture on ice and subjecting it to bright light. The zoospores, on release from the vesicle, are at first spherical to ovate (Figs. 18 C and 19 F), but over several hours become acuminate (Figs. 18 F and 19 A). At this time they average 8.6 /u in length and 3 /u in width. The flagella average 15.8 /u in length and become abruptly thinner at the tips (Fig. 18 G). They show no phototactic response, but in culture settle in greatest quantities at the air/water/glass interface nearest the light source.

The contents of six vesicles were cultured separately on agar

plates and in all cases the biflagellate zoospores gave rise to typical Codiolum plants (Figs. 18 H, I and 19 C - E). The stipe of such cells contained very regular pectic layers which increased in number with the age of the plant. On the other hand, quadriflagellate zoospores from dwarf plants or filaments never gave rise to Codiolum cells. Within thirty days of culture the Codiolum cells attained a length of 200 - 300 μ and a width of 30 - 50 μ. About 3% of these became fertile at 5°, 10°, and 15°C, but none at 20°C. In all cases fertile cells produced normal quadriflagellate zoospores which germinated within the cell. Out of this mass, filaments or dwarf plants emerged. A cold shock of one day at -5°C failed to increase fertility in vegetative Codiolum cells.

At first it was thought that the biflagellate zoospores were gametes and attempts were made to mate these with each other and with gametes of U. wormskioldii. This was done on three separate occasions, but in all cases the biflagellate zoospores showed no copulation tendencies.

Cytology and cytochemistry

Urospora vancouveriana nuclei stain similar to those of U. wormskioldii and show the same features. In small filaments and dwarf plants, the chromosomes at metaphase are very small and dot-like (Fig. 20 B). However, in early prophase the chromosome strands are readily visible (Fig. 20 C, upper left cell). In larger filaments the chromosomes at metaphase show some variation in size and shape (Fig. 20 E and H) where the largest ones are 2.5 μ long and 1 μ wide. The chromosome number, based on twelve counts, appears to be nine (Fig. 20 D - F). The Codiolum cells were uninucleate except when undergoing fertility. Only two nuclear divisional stages were observed, both in first division anaphase. A spindle apparatus was clearly observed but chromosome counts could not

be made. The cell walls of U. vancouveriana gave the same color reactions and behaved in the same way to chemical treatments as those of U. wormskioldii.

3. Type III. Urospora speciosa (Carm.) Leblond ex Hamel

In May of 1964, several small vegetative filament fragments (Fig. 21 G), less than 30 μ wide and 2 mm long, were found in a collection of Urospora wormskioldii plants obtained from the low tide region (2 ft) at Deadman's Bay (Fig. 4 G - 1). Though these fragments appeared very similar to Ulothrix flacca (Dillwyn) Thuret, which was also present, one contained fertile cells which produced zoospores lacking eyespots. The fragments became fertile in culture and ten clones were established from the resultant germlings. Of these, eight gave rise to a small form of Urospora, one to U. wormskioldii, and one to Ul. flacca. The cultural filaments of this Urospora species range from 1 - 2 cm in length and 30 - 50 μ in width. The holdfast is composed of from one to four rhizoids. The cells are mostly isodiametric and slightly swollen. The chloroplast is cylindrical, irregular at the edges, and contains several pyrenoids. Fertile cells produce quadriflagellate acuminate zoospores ranging from 8 - 12 μ in length and 3 - 4 μ in width. The cells contain a single nucleus which is readily distinguished in the living state. When stained, the nucleus shows the same features (Fig. 21 H) as those of the first two Urospora species. Other features of its life history remain to be studied. One attempt was made to induce sexuality in this form but failed (Details p. 29).

Summary of the genus Urospora

Three species of Urospora were found in this study. U. wormskioldii (n = 12) is the most common. This species is dioecious, anisogamous, has three somatic stages: filament, dwarf and Codiolum. Codiolum plants are produced by zygotes, female gametes and probably male gametes, and therefore may be diploid or haploid. The vegetative features of this species are extremely variable in the field and encompass those of other Urospora species recorded from this coast; U. penicilliformis, U. tetraciliata, U. dolifera Setchell and Gardner, U. sphaerulifera Setchell and Gardner, and U. grandis Kylin.

Urospora vancouveriana (n = 9) was found in only one locality. The filaments in nature are of a distinctive large form but in culture are indistinguishable from U. wormskioldii. This species is asexual, has three somatic stages: filament, dwarf and Codiolum. The latter stage is formed from biflagellate zoospores which are produced at higher temperatures.

Urospora speciosa (n = ?), found once at one locality is a very small slender form and, unlike any other form, is uninucleate. This is the first record of its occurrence in North America. Other features of its life history have not been studied.

4. Codiolum gregarium A. Braun and C. pusillum Lyngbye Kjellman

Ecology

Codiolum was generally found in a narrow band (1 - 1½ feet vertical depth) coinciding approximately with the higher of high tides from spring to early fall (April - September): below this level in sheltered areas (Friday Harbor, Tsawwassen) or above it in more wave beaten areas (Point No Point, Ogden Point Breakwater) (Fig. 5). It occurs densest in protected areas, on long stretches of vertical faces (Ogden Point Breakwater) and is very sparsely scattered on beaches having rocky outcrops (Point No Point, Deadman's Bay). Codiolum is frequently associated with Prasiola meridionalis Setchell and Gardner (Tsawwassen, Ogden Point Breakwater, Deadman's Bay, Stanley Park, Anacortes) where it may be found intermingled with Prasiola or above or below it. Codiolum is found in most abundance during fall and winter on flat faces of logs or rocks with a south, southwest or southeasterly exposure, above areas of maximum Urospora density. Juvenile forms appear in the spring at the upper limit of U. wormskioldii, soon after Urospora becomes sexual. The Codiolum cells continue to grow and remain vegetative during the summer months. Generally vegetative plants are most abundant and conspicuous during the fall and winter (Fig. 6). Fertility starts in the fall and reaches a peak during December and January. By following the development of small isolated patches of Codiolum at Tsawwassen it was noted that some plants may remain vegetative throughout the winter to become fertile in the next. Codiolum, as well as Urospora, may be killed off during hot summers particularly in areas with southern exposure. During the summer of 1963 at Tsawwassen, Urospora disappeared completely from the southeast side of the

jetty, whereas patches of Codiolum (0.1 - 0.5 m^2) remained, though with considerable reduction in numbers. Both survive much better on sunken logs or pilings. At Friday Harbor both plants were present on a sunken log (Fig. 28 B) throughout the summer, but absent from the rocks nearby.

Codiolum is an extremely hardy plant; more so than Prasiola, Porphyra and Bangia which are killed in the summer in protected areas. Prolonged rainy spells or freezing conditions seem to have no effect on Codiolum. In fact, in the winter of 1962 - 63 Codiolum was covered with ice for several weeks yet remained perfectly viable on thawing. Its extreme hardiness is further demonstrated by the ability of very young plants to survive three months of dry storage at room temperature (20 - 23°C). Under this condition larger plants die within two weeks.

Morphology and variability

In the ten localities in which it was found Codiolum shows a great variability (Fig. 6). The morphology, though constant for a particular locality in one year, may change in the next. It also differs according to the substrate, amount of crowding and exposure. Two extreme types are represented. The plants from Oak Bay, 1963 (Fig. 24 D) are long and slender, tapering gradually from the clava (head of the cell) to the base. These were extremely crowded and formed a dense almost pure stand on rocks (Fig. 4 E). Mature plants range from 900 - 1500 μ in length and 55 - 75 μ in width with the clava constituting about one half of the total length of the cell. This type conforms very closely to Codiolum pusillum (Lyngbye) Kjellman. The plants from Tsawwassen (1963) (Fig. 24 E) are considerably shorter, clavate or ovoid above, with the stalk sharply delimited from the clava. These plants were not nearly as crowded as those at Oak Bay. Mature plants range from 500 - 700 μ long,

80 - 100 μ wide at the clava, with a stipe 25 - 30 μ wide at the clava tapering gradually to the base, with the clava constituting from one quarter to one half the length of the cell. This type conforms closely to Codiolum gregarium A. Braun. The stipe of both types is formed of periodic pectic layers continuous with similar layers in the clava (Fig. 24 A).

In the winter of 1961 plants at Deadman's Bay were scarce and resembled Codiolum gregarium (Fig. 6). In 1963 they were very abundant and resembled C. pusillum. The plants from Victoria Breakwater in 1962 were very abundant, many resembling C. pusillum; but in 1963 they were reduced and conformed to C. gregarium. Plants on logs at Friday Harbor, Tsawwassen, and Point No Point were like C. gregarium but very reduced in the latter two localities. The logs here were drift logs cast up on the shore during winter high tides. Plants on these logs persisted into mid-summer, but later perished. Plants at their upper tidal limit are generally smaller than those at lower levels, and as with Urospora are frequently infected by fungi, especially during winter months. Curiously, Codiolum was very scarce at Point No Point in the winter of 1963 on the rocks above the area where Urospora vancouveriana was so abundant in the summer. Less than 100 cells were found from scrapings taken from many rocks. Urospora appeared to be completely absent from this area during the winter, either in filament or dwarf form, yet very dense stands of extremely large Urospora plants were found again in the summer of 1964.

Fertile Codiolum plants from seven localities all produced the typical Urospora quadriflagellate zoospore (Fig. 25 B). These are formed throughout the cell when no vacuole is present. Frequently the zoospores at the surface are arranged parallel to it. Though many cells were

examined, natural zoospore release was never observed and only rarely were empty cells encountered. Such cells always had a longitudinal split of variable length in the clava wall. A very small percentage of fertile cells could, however, be ruptured under laboratory conditions by drying at room temperature (20 - 23°C) for about eight hours and rewetting. In such cells the clava split to release the spores. Otherwise, under normal culture conditions the zoospores always germinated internally (Fig. 24 C). This occurs also in the field where, toward the end of the fertile period, one can distinguish very easily on logs the many small oval patches of young Urospora germlings that have resulted from internal development of Codiolum spore masses. The Urospora filaments found at high tide in the spring are formed from such patches.

The vegetative cell has a parietal chloroplast containing several to many pyrenoids (depending on the cell size) and a central nucleus. One or two vacuoles were occasionally observed on either side of the nucleus but this is not an obligate feature. In crowded, rapidly-growing cells the chloroplast may be coarsely-reticulate, but in mature cells it is dense and lobed inwardly.

Culture

Zoospores of fertile Codiolum plants were cultured from Deadman's Bay, Friday Harbor, Tsawwassen, Point No Point, Oak Bay and Ogden Point Breakwater. These always gave rise to dwarf plants or filaments which are indistinguishable from those of Urospora wormskioldii (Figs. 25 E - G and 27). The Codiolum plants obtained from Point No Point came from an area directly above where U. vancouveriana was found in the summer and although zoospores from these were cultured at 15°C, no biflagellate zoospore-producing-sporangia were encountered as otherwise would be

expected if the Codiolum cells originated from U. vancouveriana. Sexuality did not occur in any Codiolum-derived Urospora clones obtained from any of the localities. However, of 114 clones established from one fertile Codiolum cell obtained from Deadman's Bay, 8 produced Codiolum cells. Though these 8 clones were subsequently subcultured at 10° and 15°C none became sexual or produced Codiolum. Likewise, of 500 clones established from 50 Codiolum cells from Tsawwassen none became sexual at 15°C (Details p. 27).

Cytology and wall cytochemistry (Figs. 26, 31 B and 34)

The nucleus of the vegetative cell of Codiolum increases with cell size, attaining a diameter of 30 /u in larger plants. The living nucleus appears hyaline and contains a prominent vacuolated nucleolus (Fig. 26 P). Occasionally several smaller bodies (5 - 10) were observed in the nucleoplasm exhibiting a jiggling motion while they moved slowly and randomly in it (Fig. 26 O). In material subjected to a combined four hour daily thermoperiod (23 - 25°C) and dessication, nuclei were frequently observed with five to ten nucleoli-like structures of variable size (Fig. 26 I and J). The nucleus of natural Codiolum, like that of cultural Codiolum, fails to stain with Feulgen or Feulgen-iron-propiocarmine. When stained with Newcomer-iron-propiocarmine the chromatin is readily visible, appearing as a reticulum in the resting nucleus (Fig. 26 D). Though many thousands of Codiolum cells were examined, first divisions were encountered in only four cells. These appeared to be mitotic (Fig. 26 A - C and E). Second and third divisions appeared normal (Fig. 26 G). However, occasional cells were found which had one or two large nuclei and two or more small degenerate nuclei indicative that nuclear division in some cells is abnormal. Nuclear divisions are

synchronous and the nuclei decrease in size with further divisions. When nuclear divisions are in progress the pyrenoids do not stain. This phenomenon facilitates the search for divisions by enabling the use of lower magnification. A slight Feulgen stain is obtained after about the fourth division (16-nucleate stage) and this stain increases gradually with further divisions to zoospore formation. One cell was observed at ninth division metaphase in which 224 of the 256 possible metaphases were located (Fig. 26 H), indicating that at least 512 zoospores may be formed by a single cell. The chromosome number of Codiolum obtained from Oak Bay, Deadman's Bay, Tsawwassen, and Ogden Point Breakwater appears to be twelve, based on the highest number found at metaphase.

Similar to cultural material, natural Codiolum cells have an outer sheath of unknown composition and inner layers of pectic substances. The behavioral details of the wall components to stains and reagents are given in Fig. 31. The pectic layers give a positive test (red) with ruthenium red, and are insoluble in Schweitzer's reagent, slug cytase, or on autoclaving in 23 M KOH. They are doubly refractive (Fig. 34 D) (a property generally not attributed to pectic compounds) and are readily soluble in boiling concentrated HCl. With the latter treatment the sheath becomes much distended (Fig. 34 E) or bursts. The sheath is singly refractive; gives a negative test for chitin, pectin, fat, or cellulose; is insoluble in Schweitzer's reagent or slug cytase; is soluble in boiling HCl - $ZnCl_2$ and on autoclaving in 23 M KOH but, unlike that of Urospora, after brief boiling in concentrated HCl, is insoluble in $ZnClI_2$, Schweitzer's reagent or IKI - H_2SO_4. Both before and after boiling in concentrated HCl a banding pattern was observed in the protoplast (Figs. 35 D and F).

Induction and inhibition of fertility

Infertile Codiolum cells from Deadman's Bay and Friday Harbor were cultured at various temperatures. All mature plants (larger ones) became fertile at 5°C and 10°C, a small percentage at 15°C and none at 20°C (Table 5). Codiolum cells from Point No Point, Ogden Point Breakwater, Oak Bay and Tsawwassen behaved in a similar way.

When plants were subjected to a daily thermoperiod of 8 hrs at 18 ± 2°C, fertility was inhibited (Table 6) and when returned to 10°C it was induced. Subsequently exposure to a 4 hr thermoperiod every two days was found to be just as inhibitory. Intervals of three and four days resulted in a small percentage of plants becoming fertile and this increased with the length of interval. Plants subjected to a combined daily dessication period and thermoperiod survived much longer than those receiving a thermoperiod only. In such cultures epiphytism was markedly reduced, whereas in those receiving a thermoperiod only, the plants became overgrown with diatoms, fungi and other algae. Using the combined treatment, vegetative cells were kept in good condition on their natural substrates, wood or rocks, for four months.

The finding of Codiolum in large quantities on a log at Friday Harbor made it possible to study the effect of transplantation on fertility. Small blocks containing Codiolum were removed from the log (Fig. 28) and placed on the back and front of ladder rungs positioned at 3 ft intervals from the +12 to the -9 ft level in the intertidal. Samples were removed on the 8th, 16th, and 30th day and the percentage of fertility was determined from several hundreds of large mature looking cells. Samples were also removed from the log and from a rock at Deadman's Bay to serve as controls.

On the 8th day a small percentage of plants were fertile on the ladder rungs at levels from 0 - 9 ft while none were fertile on the log or rock (Fig. 28 E - G). On the 16th day a greater percentage of plants was fertile on the rungs and fertility extended to the lowest level. None were fertile on the rock but a small percentage was on the log. On the 30th day Codiolum was absent on several lower rungs due to their having become detached on fertility; but where present, the percentage of fertile cells was much higher than before. On the 30th day the percentage of fertility was also much higher on the log and rock but in general it was lower than that on ladder rungs below the 6 ft level. No fertile plants were observed on the ladder rung at the 12 ft level over the thirty day period. Most of the larger plants on the south side at this level died over this period but those on the north side survived. The plants at this level were about a foot above the daily high tides so would have remained dry over the entire period except when high wave action coincided with high tides. Throughout the observation period the percentage of fertility was noticeably reduced on rungs at the 6 and 9 ft level but was comparable to that found on the rock and log. Over the 30-day period the south side of the rungs at the 6, 3 and 0 ft levels became increasingly covered with filamentous diatoms, Urospora and other algae, while the north sides remained free of epiphytes. By the 30th day the filaments of diatoms on the 6 ft level rungs attained a length up to 15 cm. It is suggestive that the lower percentage of fertility on these rungs was due to the epiphyte coverage. While the water temperatures remained fairly constant over this period the aerial temperatures dropped considerably. The increase in the percentage of fertility of Codiolum on the rocks and log can be readily attributed to this atmospheric temperature drop.

5. Codiolum petrocelidis Kuckuck and Spongomorpha coalita (Ruprecht) Collins

Plants similar to Codiolum petrocelidis Kuckuck were found in abundance within the tissues of Petrocelis franciscana Setchell and Gardner in the mid-tide (2 - 7 ft) during the summer and fall of 1961 and 1963 at Deadman's Bay and Porlier Pass where Spongomorpha coalita (Ruprecht) Collins occurred in dense patches below it in the low tide (0 - 2 ft). Only vegetative cells were encountered during this period. At Deadman's Bay, C. petrocelidis was oriented stipe up (stipe directed towards the surface of the host) (Fig. 37 D) and at Porlier Pass, either up or down (Figs. 38 A, B and 39 A). On the other hand, fertile Petrocelis tissue collected from Point No Point in December, 1963 contained fertile plants of C. petrocelidis of which the majority were oriented with the stipe down (Figs. 39 E, F, and 40 E). At Porlier Pass about 50% of the plants with the stipe directed down had a lateral appendage of variable length always directed upwards (Figs. 37 A, 39 B, and 40 C). A number of cells were found in which this lateral appendage was composed internally of periodic "V"-shaped layers identical to those of the stipe proper but pointing in the opposite direction (Fig. 37 B). A number of cells were also found which clearly showed the formation of a second stipe (Figs. 38 B - 3, and 40 A, D). In the first figure only the first two layers in the new stipe are visible. At Porlier Pass cells with stipes oriented down predominated in the summer and stipes oriented up predominated in the fall. Also, material collected from Porlier Pass showed plants oriented down to be more abundant in shaded areas than in sunny areas. Septa-like structures were occasionally observed in the stipes of C. petrocelidis from Deadman's Bay (Fig. 37 C and E). However, in all three localities

cells with a solid stipe composed of "V"-shaped layers were common.

The protoplast of Codiolum petrocelidis contains a parietal chloroplast with several pyrenoids and, except at fertility, one central nucleus. In the culture studies it was found that vegetative cells became fertile at low light intensities (25 - 100 f.c.) and only in the wet filter paper cultures. Fertile cells produced ovate quadriflagellate zoospores containing a prominent eyespot. The zoospores germinated internally and later developed into branching multinucleate, multicellular filaments. Further development of these plants was not followed.

Cytochemical tests done on Codiolum petrocelidis give identical results to those of C. gregarium and cultural Codiolum from Urospora wormskioldii and U. vancouveriana (Fig. 32 B). The inner layers are pectic and the outer sheath is of an unknown composition. The septa-like structures observed in some cells dissolved readily in boiling concentrated HCl (Fig. 35 G) indicating the absence of cellulose.

Spongomorpha coalita was the only Spongomorpha species collected at Deadman's Bay and Porlier Pass. Mature sexual plants were collected at these localities from April to the end of August. A few unsuccessful attempts at mating were made in the summer of 1961. The plant was reinvestigated in the summer of 1964 to obtain information on its wall structure for comparison with the walls of Urospora.

The inner walls of Spongomorpha cells appear to be composed of cellulose and small amounts of pectic materials. As in Urospora the cells of Spongomorpha are bounded on the outside by a common filament membrane of unknown composition. The cellulose layers appear thicker than in Urospora and give a positive test with IKI - H_2SO_4 or $ZnCl_2I_2$ before or after heating in concentrated HCl or after autoclaving in 23 M KOH

(Cytochem.details Figs. 32 A and 35) cellulose layers are also doubly refractive under polarized light before and after removal of pectins. As in Urospora the cellulose layers are soluble in Schweitzer's reagent (Fig. 35 F) and slug cytase (Fig. 35 C and D), but only after boiling in concentrated HCl. The pectic material appears to be less than in Urospora for only a slight swelling occurs on treatment with IKI - H_2SO_4 and the filament remains more rigid after boiling in concentrated HCl. The cell wall gives a positive test for pectin with ruthenium red before but not after heating in concentrated HCl. The pectic materials are insoluble in Schweitzer's reagent, slug cytase or on autoclaving in 23 M KOH. The outer sheath gives a negative test for cellulose, pectin, chitin, or fat and is insoluble in Schweitzer's reagent or slug cytase. Unlike that of Urospora the sheath is insoluble in IKI - H_2SO_4, $ZnClI_2$ or Schweitzer's reagent after brief boiling in concentrated HCl and is still found present after autoclaving in 23 M KOH. However, after the latter treatment the membrane dissolves with the addition of water. The sheath of Spongomorpha appears to be much thicker than that of Urospora and may also form part of the crosswalls. On treatment with Schweitzer's reagent or slug cytase after brief boiling in concentrated HCl, crosswalls are occasionally left (Fig. 35 D). These are generally found in cells involved in branching. When the filaments are boiled in HCl - $ZnCl_2$ the inner layers dissolve first, leaving the outer sheath with crosswall "shadows" (Fig. 35 F), but with further heating these disappear as the sheath becomes further dissolved.

As a by-product of the wall studies it was discovered that Spongomorpha coalita in this area of study has operculate gametangia (Fig. 39 C). Subsequently operculation was also found in a collection of

S. coalita from Mussel Beach, in the Monterey Peninsula, California. *

IV. DISCUSSION AND CONCLUSIONS

1. Growth and sexuality of Urospora

The frequent failure of single zoospores of Urospora wormskioldii to germinate in culture is suggested to be due to the requirement of a diffusible endogenous factor required during a critical stage in germination. Concentrated spore suspensions would therefore reduce net outward diffusion of the factor, while weak suspensions would have the opposite effect, resulting in its loss. The fact that the source of the spores is related to their germination ability suggests the amount of the diffusible factor present in a spore is dependent on the past environmental history of the parent (Urospora or Codiolum). Since FeEDTA promotes zoospore germination, it is possible that the diffusible substance is iron in a weakly bound form. On the other hand, the beneficial effect of FeEDTA may be in promoting retention of the factor. If single zoospores also have a low germination frequency in the field it would explain why U. wormskioldii is not as abundant or more evenly spread as might be expected. As far as I am aware, a critical germination period has not been reported for zoospores in other algae.

In Urospora wormskioldii the male clone M2A appears to be far more sensitive to stress than the female F11. It does not grow at 20°, dies sooner with daily dessication periods, thermoperiods or both combined, and experiences bubbling of the outer sheath when subjected to daily thermoperiods. However, it was not determined if this is a common

* Collected by A. C. Mathieson (U. B. C.) in June, 1960.

feature for all male plants, or in other words, sex linked. If it is, the difference in heat tolerance of the two sexes could be used to advantage in establishing the presence of meiosis in the Codiolum stage. It could also be used to determine the percentage of haploid and diploid Codiolum plants in nature and to determine if the haploid Codiolum plants can be of either sex.

Though the gametes of Urospora wormskioldii failed to show any phototactic response over short periods, the fact that they settled nearest the light in culture indicates they have a positive phototactic response. However, since they settled at the air/water/glass interface, even when illuminated from below, it is possible that selection of this site is determined by other factors as well.

The spontaneous occurrence of sexuality in both sexes of Urospora wormskioldii, its gradual decrease in the female and abrupt loss in the male through serial subcultures suggests that internal as well as external factors are involved in the sexual response. This carry-over of sexuality probably occurs in the field and therefore might be regarded as an adaptive feature, for it would extend the sexual period and thereby increase zygote production. However, the failure of clones derived from Codiolum to become sexual indicates that, if carry-over is due to some built-up stored factor, this factor is lost in passing through the Codiolum stage.

The experiments on sexuality indicate that temperature change is the prime factor involved in the sexual response and that, in culture, nutrient factors are involved as well. Desiccation by itself does not appear to be important in promoting sexuality. Cyclic thermoperiods may not be a requirement for sex induction since a prolonged thermoperiod was found to be more effective. The proposal that temperature is the prime

sex-inducing factor agrees with the observation that sexual plants are found only in the summer and only in the mid-tide, and that plants with a southern exposure become sexual before plants with a northern exposure.

The spontaneous occurrence of Codiolum in cultures of male Urospora wormskioldii from three widely different localities, and its absence in serial subcultures at the same temperature suggests that this is a natural and not a cultural phenomenon. In order to account for the origin and production of Codiolum cells in original but not subsequent sexually induced cultures, it is suggested that under ideal culture conditions male gametes can develop parthenogenetically. However, since male gametes from nature or culture die when cultured free of Urospora, and since the culture medium in original and subsequent cultures was the same, it is suggested that the parthenogenic development of male gametes is dependent on a substance (or substances) produced by Urospora plants and that the substance ceases to be produced in subsequent subcultures.

2. Growth and fertility of Codiolum

The poor growth of Codiolum in culture is hard to explain in view of its small size in nature and in view of the luxuriant growth of Urospora under the same cultural conditions. Reduced growth of cultural Codiolum was also obtained by Jorde (1933) and Kornmann (1961 b, c). The decrease in cell size with decrease in temperature implies that growth is dependent on temperature. The absence of fertility in all male and female Codiolum cells of U. wormskioldii grown in liquid culture and its occurrence in female F3 Codiolum cells grown on agar medium suggests that the agar medium is a more favorable environment for Codiolum. However, this may not be true since cells on agar were invariably smaller than those in liquid culture. Since Codiolum is found only in the high tide,

it is reasonable to suppose that daily periods of desiccation and heat may be required for normal growth.

The studies of fertility in natural Codiolum show clearly that fertility is inhibited by daily warm thermoperiods and induced by cold conditions. That large cells become fertile first indicates a degree of maturity is required before fertility can be induced. The data also suggest that fertility commences about four to six days after the beginning of the cold treatment. This lag in fertility would be of survival value in areas where summer water temperatures approach 20°C for it would ensure that plants become fertile only under relatively stable cool atmospheric conditions as would occur with the approach of winter when water temperatures reach more tolerant levels for Urospora growth. Since fertile plants were never encountered in the field at lower levels, or at the lower limit of Codiolum, it is proposed that the lower limit is the lowest level at which gametes or zygotes settle, and that these settle only during high tide periods.

The results from cultural studies are not as clear cut, making interpretations difficult. The absence of fertility at 20°C is probably due to inhibition by temperature, and its absence at 5°C to poor growth and immaturity. The failure of cold treatment following culturing at higher temperatures or following daily thermoperiods suggests that either the difference in temperatures was not great enough or that factors besides temperature are involved in the fertility response. The former is probably the case since a very high percentage of fertility was obtained in cold-shocked Codiolum cells of U. wormskioldii female clone F8. That other factors are involved is indicated by the observation that the same cold-shock treatments failed to induce fertility in Codiolum from isolate URX1.

If fertility is dependent on a temperature drop and on plant maturity, this would explain why some plants remain infertile throughout the winter months. Since sexuality may occur on into September, Codiolum plants initiated at this time would develop under much colder conditions than those developing in the summer. These would experience a far smaller temperature drop which, during mild winters, might not be sufficient to induce fertility, and so the plants would persist to the next winter.

The abnormal products of fertile Codiolum cells from female clones F3 (not cold shocked) and F8 (cold shocked) appear to be due to internal factors. These were probably not due to cultural conditions since Codiolum from Urospora vancouveriana grown in liquid or agar media, always produced normal zoospores. These abnormalities would be understandable if meiosis was an obligate feature of haploid or diploid Codiolum cells in U. wormskioldii. However, if this were true, one would expect to find similar abnormalities in natural Codiolum and these were never encountered.

3. Cytology of Urospora and Codiolum

The fibrils in the walls of Urospora and Codiolum zoospores have not been described before. According to Areschoug (1874), Printz (1932), and Frye and Zeller (1915) the flagella in Urospora zoospores originate at the anterior end of the four zoospore ridges. However, in the present study, squash preparations of zoospores showed clearly that the flagella originate between the fibrils of the ridges, and hence between the ridges themselves. Since the ridge fibrils were found in zoospores of U. wormskioldii, U. vancouveriana and their Codiolum stages, it is possible that they are a general feature of Urospora. It would be of interest to determine if this feature is also present in the acuminate quadriflagellate

zoospores of other members in the Ulotrichales (sensu Kornmann). It would also be of interest to determine if the fibrils have any relation to flagellar roots as found in quadriflagellate zoospores of Ulothrix sp. (Manton, 1952) and other green filamentous algae (Manton et al, 1955; Manton, 1964). According to these authors, the flagellar roots arise between the flagella as do the surface fibrils in zoospores of Urospora.

The morphology of the nucleus and nuclear division in Urospora are essentially the same as in closely related genera e.g. Ulothrix, Spongomorpha and Acrosiphonia. The chromosomes in U. wormskioldii and U. vancouveriana are comparable in size to those of A. spinescens (Jónsson, 1962) but much smaller than those of Ulothrix (Sarma, 1963) and Spongomorpha (Jónsson, 1962). In the latter two genera some chromosomes may measure 5 μ in length, whereas in Urospora they seldom are 2 μ. Chromatic bodies occurring in Urospora were not reported in the interphase nucleus of the other species.

The decrease in Feulgen-stainability of larger nuclei of Urospora and Codiolum may be due to properties of the nucleus itself or to a spreading-out of the chromatin. The evidence for the latter possibility is that, with increasing nuclear size the Feulgen-staining chromatin strands become finer and more distantly separated until they become invisible. The evidence for the former possibility is that the chromatin appears quite dense when stained with Newcomer-iron-propiocarmine. It may be that some substance inhibits Feulgen staining in larger nuclei. If the chromatin does continue to increase in amount proportional to the increase in nuclear volume, it is curious that the chromosomes in early divisions of Codiolum are so small. Though many cells were observed with nuclei of up to 30 μ in diameter, early-division chromosomes seldom

exceeded 3 μ in length. By contrast, nuclei of Urospora, 10 μ in diameter, produced chromosomes up to 2 μ in length.

The concentration of nuclei at the end walls and in the middle of the cell in rapidly growing filaments or Urospora wormskioldii and U. vancouveriana suggests that nuclear migration takes place and that cell division is intimately related to nuclear concentration. A similar distribution of nuclei was observed in U. mirabilis by Jorde (1933). Cell division, however, appears to be unrelated to nuclear division for the two occur independently, at least in multinucleate cells. This may not be true in germlings where the cells are initially uninucleate. Nuclear migration has also been reported in Acrosiphonia spinescens (Jónsson 1960, 1962). In this species some of the nuclei migrate to the plane of the future crosswall where they divide synchronously. More distant nuclei remain quiescent. The crosswall is formed directly after nuclear division by centripetal growth. In Spongomorpha lanosa, cytogamy and karyogamy are intimately linked (Jónsson 1962). The mode of cell division in U. speciosa has not been studied.

4. Variability and Speciation in Urospora

The wide range in morphology exhibited by vegetative filaments of Urospora wormskioldii in nature places much doubt on the use of purely vegetative characteristics for dilineation of Urospora species, as has been done by Setchell and Gardner (1920) for species from this coast. This may apply as well to species from Europe which show similar variability (Printz 1932, Jorde 1933). In view of this, the taxonomy of the genus, to be meaningful, must be based on sexual, life history and cytological features as well as morphological ones.

At the present time, six taxa of Urospora may be recognized from

Europe; U. bangiodes, U. mirabilis, U. wormskioldii var wormskioldii, U. wormskioldii var biflagellatum, U. wormskioldii var caudatum and U. speciosa. The first, second, fourth and sixth taxa appear to be distinct entities; however, the remainder are questionable. U. wormskioldii var wormskioldii, a low tide form, has not been found sexual in Europe. According to Jorde (1933) it may be merely a growth form of U. mirabilis. According to Printz (1932), U. bangiodes is synonomous with U. mirabilis, while Kornmann (1961c) considers it to be a distinct species since it differs from others in that cultural plants have a single richly branched rhizoid. Kornmann (1961c) considers U. bangiodes and U. wormskioldii var caudatum to be asexual since these were never found sexual in the field or when cultured at various temperatures (3° - 15°C). Both reproduce via quadriflagellate zoospores which, in the latter taxa, are also capable of giving rise to a Codiolum stage. His evidence for the origin of Codiolum is, however, indirect and therefore questionable. To establish this point the development of zoospores would have to be followed. It is equally possible that the Codiolum cells arose from a small number of gametes which could have gone undetected in culture. Kornmann's conclusion that both species are asexual is also questionable since the same temperatures failed to induce sexuality in U. speciosa and U. penicilliformis which were found sexual in nature. In the present study several clones derived from sexual and vegetative filaments of U. wormskioldii behaved in a similar manner; some failed to produce Codiolum while others did in amounts ranging from one to over 500 per test tube. Unless parthenogametic development of gametes is very low in culture very few gametes would be required to produce these results. The appearance of Codiolum cells in 8 out of 114 Urospora clones derived from a Codiolum cell from Deadman's

Bay and none in the 500 clones derived from 50 Codiolum cells from Tsawwassen can be used as specific examples. In the first case, though the 8 clones were recultured at 5°, 10° and 15°C, none became sexual or produced Codiolum. Since only U. wormskioldii was present at Tsawwassen and most abundant at Deadman's Bay, it is very likely that the Codiolum cells used to establish these cultures were derived from sexual U. wormskioldii. Therefore failure to obtain sexuality in culture over the temperature ranges used cannot be taken as proof of asexuality. Of particular interest is the clone URX1 (a low tide form which I attribute to U. wormskioldii). This clone has been recultured many times over the past three years and, in two instances, produced Codiolum cells in great abundance when cultured at 10°C; once after twelve months of culture and once after twenty months. On the last instance this occurred in a growth experiment involving various media. Of sixteen replicate cultures only one of these produced Codiolum, this occurring in medium 4 (Table 2). Subsequent cultures in the same medium failed to produce Codiolum. Though zoospores from this clone were cultured on liquid and agar media at 5°, 10° and 15°C all developed into dwarf plants or filaments. The failure of URX1 to become sexual with any of the methods used in the present study might indicate it to be a different species to U. wormskioldii - perhaps an asexual one. However, further evidence is needed to be sure of this point.

Though it is reasonable to expect that asexual species of Urospora exist in nature, means to obtain convincing evidence for proof of asexuality at present are not available. Transplantation studies might be usefully applied to this problem. Since Urospora is small and easily cultured in large quantities, cultural material could be

transplanted to areas in the intertidal where and when sexuality occurs in other species. Transplantation techniques might also be used to study the effect of habitat and seasons on growth and morphology of Urospora. In particular these would be useful in determining the nature of "asexual" low tide forms. To facilitate this type of work, an easily-handled substrate and labelling technique would be required. Ceramics might be useful as a substrate since any shape or texture could be made. The ferric chloride-potassium ferrocyanide marking technique used by Astbury and Preston (1940) on Cladophora or Calcofluor White in conjunction with fluorescent microscopy (Cole, 1964) might be useful for labelling and later identification.

The names applied to the three Urospora species found in this study are based on Setchell and Gardner's Key (1920) and are considered tentative. These species show close resemblance to European types but, because of incomplete or conflicting data, evaluation is not possible. Type I designated as U. wormskioldii conforms very closely in gametangium color and gamete size to U. penicilliformis from Helgoland (Kornmann, 1961c), but differs from it in that the male gametes are spindle shaped, the female gametes are asymmetrical and the male as well as the female has a pyrenoid (Fig. 22). In U. penicilliformis both gametes are ovate and only the female has a pyrenoid. However, these distinctions may not be valid. In U. wormskioldii the small pyrenoid of the male gamete is difficult to distinguish unless stained and the asymmetry of the female gamete is not assumed until some time after release. Kornmann did not stain his material nor did he observe gametes over long periods (personal communication). Future investigations of gamete morphology should take the time element into consideration. Since sexual material of U.

wormskioldii has not been found in Europe, a comparison with it is not possible. Type I appears to be different from U. mirabilis in gamete morphology and chromosome number. However, the description of the gametes of U. mirabilis given by Printz (1932) is at variance with Jorde's description and drawings (Fig. 22). Furthermore, the chromosome number of four for U. mirabilis was given by Jorde with some reservation so that a meaningful comparison cannot be made. However, since both gametes of U. mirabilis have an eyespot which is present only in the male of Type I, it would indicate that each is a distinct species.

On the basis of information presently available on life history and morphological features in Urospora I am inclined to recognize five species from the Pacific Coast of North America; U. speciosa, U. vancouveriana, U. wormskioldii, U. tetraciliata and U. penicilliformis. However, the last two are considered doubtful entities, since the unusual nature of the gametes in U. tetraciliata has not been confirmed and since sexuality has, as yet, not been recorded in America in U. penicilliformis*. U. dolifera, U. grandis and U. sphaerulifera are not regarded by me as distinct entities since they are known only from vegetative material and they come within the range of U. wormskioldii. With these reservations the following key is proposed as a tentative one for Urospora species which I recognize from this coast.

* Life history studies on Urospora penilliformis might be profitably done in the Monterey Peninsula since only this species is recorded from that area (Smith, 1944).

Key to Urospora species

1. Uninucleate; monoecious; isogamous; filaments under 60 μ wide: 1 - 4 extramatrical rhizoids _____ U. speciosa

1. Multinucleate; rhizoids intra or extramatrical filaments up to 3 mm wide _____ 2

2. Sexual _____ 3

2. Asexual; Codiolum arising from biflagellate zoospores produced by dwarf plants at warm temperatures; filaments up to 3 mm wide _____ U. vancouveriana

3. Monoecious; gametes quadriflagellate, isogamous; filaments up to 225 μ wide _____ U. tetraciliata

3. Dioecious; gametes biflagellate, anisogamous, gametes ovate, male gametes smaller; filaments under 100 μ wide _____ U. penicilliformis

3. Dioecious; gametes biflagellate, anisogamous, male gametes smaller, spindle shaped; female gametes asymmetrical, slightly curved, twisted and having 2 or more blunt longitudinal ridges; filaments up to 1200 μ or more in width _____ U. wormskioldii

Type II designated as Urospora vancouveriana is very similar to Kornmann's (1964 b, c) U. wormskioldii var biflagellatum from Helgoland, with this similarity extending to the temperature requirements for the production of the biflagellate zoospores. These are of similar size in both species but in U. wormskioldii var biflagellatum they are ovate

whereas in U. vancouveriana they are acuminate (Fig. 22). This difference may not be meaningful since Kornmann did not study the biflagellate zoospores over lengthy periods. Kornmann has observed zoospores with pointed ends which were not mentioned in his paper (personal communication), suggesting that a similar change may take place in the biflagellate zoospores of this species over time. The main difference in the two species is that the fertile zoosporangia, producing biflagellate zoospores, in U. wormskioldii var biflagellatum are warty at the apex but smooth in U. vancouveriana. Comparison of the two on filament characteristics is not possible, since filaments of U. wormskioldii var biflagellatum have not been found by Kornmann in nature.

Type III is designated as Urospora speciosa, because of its close resemblance in vegetative features to that species at Helgoland as described by Kornmann (1961c) and because the Helgoland type is also uninucleate (Kornmann, personal communication). Further life history information is needed for the American type, however, before the two can be equated.

The discovery of a uninucleate species of Urospora is further evidence for the evolution of Urospora from Ulothrix. According to the precedent set by Wille (1900) for separating genera on the basis of nuclear condition, a new genus could be erected for uninucleate Urospora types as well. In view of the confusion that has resulted by creating new genera in the Acrosiphonia-Spongomorpha complex, it should be avoided until cytological as well as life history information is available for other species.

5. Variability and Speciation in Codiolum

The variability in the morphology of natural Codiolum appears

to be due to environmental conditions. Similar variability was observed for European forms by Printz (1932) and Jorde (1933), who considered these to be merely variants of C. gregarium A. Braun. The two types encountered in the present study, C. gregarium and C. pusillum, are considered to be synonymous and to belong to the life history of U. wormskioldii for the reasons that: they were found only in areas where U. wormskioldii was most abundant; they have the same chromosome number as U. wormskioldii; and Urospora clones derived from them, when cultured at 15°C, behaved like U. wormskioldii. However, it obviously cannot be concluded that free living Codiolum forms in other localities belong to this species. This would have to be determined from cultural and cytological studies.

Codiolum petrocelidis is considered to belong to the life history of S. coalita because of its close association with that species. According to Fan (1959), C. petrocelidis is multinucleate. In this study the plant was found uninucleate from April through to September, only becoming multinucleate during the fertile period in the winter. Fan's observations of the nuclear condition seem to have been on cultural plants alone so that his results are not to be equated with conditions in the field.

The different forms and orientations of Codiolum petrocelidis cells observed in this study are best explained by assuming that the cells are capable of reversing their direction of growth. The direction of growth is suggested to be governed by light intensity, a high intensity promoting downward growth of the protoplast and low light intensity, upward growth. This would be in accord with the observation that plants at higher latitudes (Helgoland 54°N) grow mainly stipe down and plants at lower latitudes

(Monterey, California 36°N) grow stipe up. This hypothesis could readily be tested with culture or transplantation studies. Growth reversal would also explain the significance of the lateral stipe appendage observed by other authors e.g. Kuckuck (1894), Zimmerman (1925), Printz (1926), Kornmann (1961a). (Their drawings are brought together in Fig. 36 for comparison purposes). The appendage would merely represent the first-formed stipe. There are two facts favoring this proposal: the appendage is always directed upwards and its "V" shaped lamellations point in the opposite direction to those in the stipe proper.

According to Zimmerman (1925) the lateral appendage is a secondary outgrowth resulting from the swelling of the wall where the wall is poorly attached to the cell. It is not clear whether he was referring to the outer sheath or inner walls, but the former seems the case. Jónsson (1962) concluded that in Brittany, France, C. petrocelidis changes its direction of growth, but in so doing reabsorbs the first formed stipe. In the latest stage the first stipe is represented by a thickened apical cap (Fig. 36 D - f). Jónsson's conclusion is based mainly on development of zygotes of Acrosiphonia spinescens in culture; the figures from nature shown here being arranged to conform with cultural observations. Jónsson, however, did not suggest any reason for the change in growth.

In order to obtain cells with appendages one would have to assume that in this area reverse growth occurs by the protoplast bulging out laterally and then bypassing the stipe. The fact that the lateral appendage can be found anywhere on the stipe indicates that growth of the outer membrane about the clava is diffuse. Otherwise, if growth occurred only at the advancing end of the protoplast, the lateral appendage would

always be located at the base of the main stipe.

Though the descriptions of the morphology of C. petrocelidis are at variance in regards the stipe, to date all investigators, except Kornmann, regard the plant to be unicellular. According to Kornmann (1961a), the septa-like structures he observed in developing cultural zygotes of Spongomorpha lanosa (Fig. 36 G) and those observed by Fan (1959) in developing zygotes of S. coalita (Fig. 36 F) reflect an early multicellular condition. However, though Fan did not follow this development in detail, he clearly stated that the cells are unicellular. Hollenberg (1958), who worked on S. coalita from the same area as Fan, found developing zygotes to have an aseptate stipe (Fig. 36 H) indicating that septation is not an obligate feature. Jónsson (1958), who observed similar septa-like structures in zygotes of Acrosiphonia spinescens (Fig. 36 E), regards these as pseudosepta (personal communication). Kornmann did not follow development of C. petrocelidis in the field but agrees that in nature C. petrocelidis is unicellular (personal communication). The septa-like structures found in the present study are regarded to be merely the result of uneven deposition of pectic layers in the stipe. It is also suggested that the mode of stipe growth (deposition of pectic layers by the protoplast) rules out a multicellular condition. However, since different species are involved, Kornmann's interpretations cannot be ruled out. A careful cytological study on the early development of zygotic material from Helgoland is therefore urgently needed.

6. Cytochemical studies

The cytochemical studies have shown that there are qualitative and quantitative differences in the walls of Spongomorpha and Urospora, but not in the walls of their respective Codiolum stage. All four forms have

an outer membrane possessing the common characteristics of being singly refractive under polarized light, insoluble in slug cytase, concentrated HCl, or Schweitzer's reagent, and of giving a negative test for cellulose, chitin, pectin, or fat. Three types of membranes appear to be involved since only that of Urospora dissolves in $ZnClI_2$ or Schweitzer's reagent after brief heating in concentrated HCl, and only that of Spongomorpha is insoluble on autoclaving in 23M KOH. The inner walls of Urospora and Spongomorpha differ in that pectic compounds seem predominant in the former and cellulose in the latter. The inner walls of the Codiolum types are pectic and lack cellulose. The pectic component in all forms appears to differ from that of higher plants, a feature also noted by Astbury and Preston (1940) for the pectic component in the walls of Cladophora.

Some aspects of these results are in conflict with those of Jónsson (1962) and earlier authors. Braun (1855) reported that the outer membrane of Codiolum gregarium gave a dark color with IKI - H_2SO_4, which changed to green, but never blue, on addition of $ZnClI_2$. I could not verify this color change. Zimmerman (1925) obtained a slight blue color in the anterior part of the cell with IKI - H_2SO_4, otherwise the outer membrane remained unstained. He found this membrane gave no stain with methylene blue or congo red and was insoluble in the cellulose solvent, cupric-ferric-ammonium hydroxide and that the inner wall material dissolved readily in concentrated sulphuric acid. Zimmerman concluded that, in addition to a small amount of cellulose, the membrane is composed of some other material but not pectin. Jónsson (1962) failed to obtain a positive test for cellulose with $ZnClI_2$ in Spongomorpha, Urospora, Acrosiphonia, and also Cladophora, Rhizoclonium, and Chaetomorpha. In all of these he reported the cellulose fraction to be insoluble in Schweitzer's reagent

and therefore different from that of higher plants. However, it appears that he did not pretreat for removal of pectic compounds. It has been demonstrated in the present study and much earlier by Wurdack (1923) and Tiffany (1924) that pectic compounds act as a barrier to this cellulose solvent. According to Jónsson (1962) the outer membrane in Urospora, Spongomorpha and Acrosiphonia contains pectic materials, since he found it to stain with ruthenium red. However, this membrane is so thin that coloring in adjacent layers might be attributed to it. If pectin is a component it would have to be present in small quantities, since the membrane does not change noticeably in thickness after treatment for removal of pectins.

A similar non cellulosic type of membrane has been described occurring in Cladophora (Brand 1901, Wurdack 1923), Bulbochaete (Tiffany 1924), and Oedegonium (Hirn 1900, Wurdack 1923). Cytochemical tests indicated the membrane to be chitin in Cladophora and Oedegonium (Wurdack 1923) and Bulbochaete (Tiffany 1924). Astbury and Preston (1940) confirmed the presence of chitin in Cladophora, using cytochemical and X-ray diffraction techniques, but did not localize it. However, more recently the existence of chitin in Cladophora has been placed in doubt. Frey-Wyssling (1959), using X-ray techniques, and Jónsson (1962), using in addition cytochemical techniques, failed to demonstrate its presence in Cladophora. Though no conclusion can be made concerning the composition of the membranes as reported, it appears that a substance different to cellulose and chitin is involved.

The banded appearance of the protoplast of Codiolum gregarium seen under polarized light, both before and after treatment with HCl, suggests the presence of oriented structures within the cell. The presence

of "microtubules" as found in root tip cells of several higher plants (Ledbetter and Porter, 1963) would provide an explanation for this banding. It has been suggested by these authors that the "microtubules" may be related to the deposition and orientation of cellulose in the cell wall, since a correlation has been found in the orientation of cellulose fibres and the "microtubules". This hypothesis would not apply here, however, for the sheath and inner walls of Codiolum appear to be non-cellulosic.

Differences in wall composition as revealed by X-ray diffraction techniques have been used for taxonomic purposes. Jónsson (1959b) used his X-ray data as supporting evidence for the inclusion of Urospora, Spongomorpha and Acrosiphonia in his new family, the Acrosiphoniaceae, while Kornmann (1963) uses the data of Nicolai and Preston (1952) as supporting evidence for the inclusion of Urospora, Ulothrix, Gomontia and Monostroma in the Ulotrichales (sensu Kornmann) and the exclusion of Acrosiphonia and Spongomorpha from this order. However, interpretations of X-ray data obtained from studies on untreated bulk material, as done by these authors, have been questioned. Frey and Preston (1961) found that in a number of cases the ambiguous results obtained from some algae in the early work of Nicolai and Preston (1952) were due to contamination of clay minerals. Also, Cronshaw, Meyers and Preston (1958) have shown that X-ray diffraction patterns are influenced by other wall components. The results obtained in this present study indicate the walls of Urospora and Spongomorpha to be different but the walls of their respective Codiolum stages to be the same. These results at once imply both a closer and more distant relationship of the two genera. Clearly, wall studies on algae having hetermorphic life histories should include all

stages to be of full taxonomic value.

7. The Acrosiphonia-Spongomorpha complex.

The taxonomy of the Acrosiphonia-Spongomorpha complex has been in a confused state for some time. Part of this confusion is due to Wille (1900), who proposed the retention of the two genera and the relegation of uninucleate types to Spongomorpha and multinucleate types to Acrosiphonia. Setchell and Gardner (1920) preferred not to draw a distinction for West Coast North American types until cytological information was available. Smith (1946) rejected Wille's proposal completely, feeling that aside from nuclear condition the two genera agreed in all other respects. Jónsson (1957) was the first to discover operculation in Acrosiphonia and since then Kornmann (1962) has come to regard it as a distinctive feature of Acrosiphonia. However, Jónsson also accepts its uninucleate condition as a distinctive feature. On the other hand, Kornmann rejects Wille's proposal, pointing out that multinucleate as well as uninucleate species exist in Acrosiphonia. He gives no examples but it appears he is referring to S. coalita from America (Fan 1959). In addition to operculation, Kornmann, on the basis of his studies, recognizes an isomorphic life history as the distinctive feature of Acrosiphonia and a heteromorphic life history as the distinctive feature of Spongomorpha. Kornmann would therefore claim S. coalita as a true member of the genus Spongomorpha. Jónsson (1957, 1959a, 1962, 1963), however, claims Acrosiphonia in Brittany, France to have a heteromorphic life cycle and, more recently (1964 a, b), has presented additional evidence to support this claim. Jónsson points out that Kornmann's failure to obtain Codiolum from zygotes of A. arcta may be due to a complete failure of karyogamy since Jónsson reported that karyogamy in A. spinescens (= arcta,

Kornmann 1962) is facultative. According to Jónsson, when karyogamy fails, ordinary Acrosiphonia plants are produced and when it occurs Codiolum is produced. Recently, Kornmann has come to consider A. arcta at Helgoland to be a diplont (personal communication) but gives no cytological evidence for this.

The discovery of operculation in Spongomorpha coalita now makes it possible, at least for the present, to clear up this area of confusion. Since both Jónsson and Kornmann agree that operculation is a distinctive feature of Acrosiphonia then S. coalita should probably be relegated to the genus Acrosiphonia. Doing this would mean recognition of a heteromorphic life history for Acrosiphonia, and acceptance of Wille's basis for distinguishing the two genera since S. coalita is multinucleate and has a heteromorphic life history (Fan 1959). Formal transfer, however, should await examination of the type specimen.

Until more convincing evidence is brought forth, the sporophyte of Spongomorpha should be considered unicellular. Recognition of this and a heteromorphic life history in Acrosiphonia re-establishes the basis for Jónsson's family, the Acrosiphoniaceae, and, at the same time, removes the main barriers to the inclusion of Acrosiphonia and Spongomorpha in the Ulotrichales (sensu Kornmann). The present author, however, is of the opinion that Urospora should not be placed in the Acrosiphoniaceae because of its asexual reproduction by means of acuminate quadriflagellate zoospores.

Conflicting reports concerning the life cycle of Acrosiphonia arcta (= A. spinescens) at Helgoland (Kornmann) and Roscoff, France, (Jónsson) may indicate that a greater variability and flexibility in life histories occur in this complex than has been suspected. It is clear that future life history studies in this group should be very

critical. The discovery of facultative karyogamy in Acrosiphonia by Jónsson (1962, 1964 a, b) emphasizes that cytology should form an integral part of such studies. Investigation of North American species in the Acrosiphonia-Spongomorpha complex should be particularly rewarding, since six species of Spongomorpha are recorded from British Columbia, and northern Washington (Scagel, 1957) and four species from the northeastern coast of America (Taylor, 1957).

8. Theoretical considerations on the origin of Codiolum

According to Jorde (1933), the diploid Codiolum stage of Urospora can be considered as an advanced zygote. Though she gives no evidence to support this view, it is put forth, presumably, on the basis that Codiolum exhibits growth over a prolonged period. These studies demonstrate that Codiolum may have a life span of over six months, and that during this period growth may be continuous. Certainly, the Codiolum stage cannot be viewed as a resting zygote as is found in fresh water algae. Jónsson (1962), on the other hand, considers the Codiolum stage in Urospora, Spongomorpha and Acrosiphonia to be a much reduced sporophyte and suggests that in the latter two genera this reduction has come about as an adaptation to an endophytic habitat. However, as he states, this explanation loses some of its value when one considers that the sporophyte of Urospora is never found endophytic, though it is just as reduced in form. In support of his hypothesis, Jónsson draws from information on life histories in Stigeoclonium. In Stigeoclonium subspinosum the sporophyte consists of a few cells, whereas in Stigeoclonium amoenum it is a single cell. (Juller, 1937). Jónsson's reductional hypothesis is in keeping with the hypothesis put forth by Fritsch (1942) that heteromorphic life histories in the algae have arisen from isomorphic ones.

The hypothesis that the diploid state in algae has arisen by prolongation and further development of a zygote (Smith, 1938) could be applied here as well. In this case competition for substrate light and nutrition would be major factors affecting the survival of the zygote. This would be true, however, only on the assumption that a rich algal flora existed at the time when the green filamentous algae were undergoing evolution from a haplontic to a haplodiplontic way of life. In meeting this competition the newly formed zygote could take one of several steps to ensure its retention in the life cycle. The zygote could become fertile, soon after formation, to regenerate the haploid form. Nuclear division could take place to give rise to a coenocyte, or cell division could take place to give rise to a multicellular form different or identical to the haploid generation. Lastly, the zygote stage could be prolonged. In the last instance, competition would be very great unless a site were selected where competition could be avoided. Urospora, Acrosiphonia, Spongomorpha, Gomontia and Monostroma all possess the unique feature that their Codiolum stages occupy habitats where competition is virtually absent. The extreme conditions of the high tide habitat of the Codiolum stage of Urospora is tolerated by few algae. The endophytic habitat of the Codiolum stage of Acrosiphonia and Spongomorpha, in various red algae is protective. Similarly the burrows of the Codiolum stage of Gomontia and Monostroma in mollusc shells are protective. It is therefore equally reasonable to suggest that the heteromorphic life cycle in these genera arose directly from haplont ancestors.

While either hypothesis may be true, adherence to the first, that a heteromorphic life cycle arose from an isomorphic one by reduction, would require explanation as to why the diploid rather than the haploid

generation should have been reduced in these five closely related genera and why this reduction went so far. If Kornmann is correct, that Acrosiphonia arcta at Helogland is a diplont and that the zygote of S. lanosa in culture is multicellular, this would provide convincing evidence for Jónssons reductional hypothesis.

V. FINAL SUMMARY

The distribution, morphology, cytology and cultural behavior of Urospora and Codiolum (free living and endophytic) have been studied from a number of localities within a radius of approximately 100 miles of Vancouver, British Columbia.

Urospora wormskioldii (n = 12) is the most common species, ranging from low to high tide levels, being densest in the mid-tide. It is dioecious, anisogamous and has three somatic stages, dwarf, filamentous and Codiolum. The vegetative characteristics of the filament stage are extremely variable and depend on the substrate, locality and season. Cultural plants show a similar variability, though not as great. The Codiolum stage arises from zygotes, female gametes and probably male gametes. C. gregarium and C. pusillum, as found in the areas studied, are considered to be merely form variants and to belong to the life history of U. wormskioldii. Fertility in natural Codiolum is inhibited by short daily thermoperiods and induced by cold conditions. On fertility, natural and cultural Codiolum produce quadriflagellate zoospores which give rise to the filamentous or dwarf stage. The last two stages reproduce asexually via similar zoospores. Sexuality occurred spontaneously in many Urospora clones cultured at 10°C., continuing through several serial cultures of female filaments but stopping after the first culture of the male filaments. Following loss of sexuality in culture, a long

thermoperiod during maximum filament growth followed by nutrient repletion successfully induced sexuality in several Urospora clones. Nuclear division was followed in natural Codiolum but meiosis was not demonstrated.

Urospora vancouveriana (n = 9) is a large low tide form and was found in only one locality. It is an asexual species having filamentous, dwarf and Codiolum stages. Cultural filaments are indistinguishable from cultural ones of U. wormskioldii. All three stages produce quadriflagellate zoospores which form either dwarf plants or filaments. In addition, dwarf plants when cultured at warmer temperatures, e.g. 15°C, produce biflagellate zoospores which give rise to the Codiolum stage.

Urospora speciosa (n = ?) is a very slender form known from a few filament fragments obtained from the low tide at one locality. Filamentous and dwarf stages are present, both of which reproduce via quadriflagellate zoospores. Other features of its life history are unknown. U. speciosa was discovered to be uninucleate, this being a unique feature in the genus. Since Urospora dolifera, U. grandis and U. sphaerulifera are known only from vegetative material and since these species come within the range of U. wormskioldii they should be regarded as doubtful entities. The discovery of a uninucleate condition in Urospora speciosa provides further evidence for the hypothesis that Urospora evolved from Ulothrix. Rather than create a new genus, it is suggested that this be avoided until cytological data for other species becomes available.

Cytochemical tests were conducted on the walls of Urospora wormskioldii, U. vancouveriana and their cultural Codiolum stages; C. gregarium, C. pusillum, C. petrocelidis and Spongomorpha coalita. The results indicate the inner cell walls of Urospora and Spongomorpha are composed of cellulose and pectic material, with pectic materials

predominating in the former and cellulose in the latter. The inner walls of their respective Codiolum stages are pectic. The pectic material in all forms appears to be different to that of higher plants. The outer ensheathing membrane of Urospora, Spongomorpha and the Codiolum types is composed of an unknown substance which gives a negative test for cellulose, pectin, chitin or fat. Three types of membranes appear to be involved, of which the Codiolum types are the same.

The differences found in the cell walls of Urospora and Spongomorpha indicate a more distant relationship of the two genera, while the similarity of their Codiolum stages indicates a closer one. Obviously, to be of taxonomic value, wall studies should include all stages in life histories of algae having an alternation of heteromorphic generations. These studies also point to the need for re-examination of other green filamentous algae reported to have an outer membrane of different nature to cellulose.

In the areas investigated, Codiolum petrocelidis, found as an endophyte in Petrocelis franciscana, is suggested to belong to the life history of Spongomorpha coalita. Morphological and developmental features of the cell are described. The protoplast of C. petrocelidis may reverse its direction of growth. The direction of growth is suggested to be governed by light intensity. Accordingly, the mode of growth rules out a multicellular condition for C. petrocelidis.

Spongomorpha coalita from this area and from the Monterey Peninsula, California where the major investigations on this species have been conducted, is reported for the first time to have operculate gametangia. This discovery supports a transfer of this species to the genus Acrosiphonia. However, formal transfer of the entity should await comparison with the type specimen. This transfer would validate a previous

distinction based on nuclear condition. At the same time, it would verify the occurrence of a heteromorphic life history in the genus Acrosiphonia.

Until more convincing evidence is presented, the zygote forms of Spongomorpha and Acrosiphonia must be considered unicellular. This removes the main objection to the inclusion of the two genera in the Ulotrichales (sensu Kornmann) and, at the same time, re-establishes the basis for Jónsson's family, the Acrosiphoniaceae.

Consideration is given to the possible origin of the Codiolum stage from a haplontic life history as an alternative to Jónsson's (1962) reductional hypothesis.

BIBLIOGRAPHY

Anon,(1962 - 3). Pacific Coast Tide and Current Tables 1963 - 4. Can. Hydrographic Service Publ. Queens Printer, Ottawa. 1962, vi 262 pp; 1963, vi 280 pp.

Archer, A. and E. M. Burrows (1960). Heteromorphic life - history as a family criterion in the Cladophorales. Brit. Phyc. Bull. 2:31-33, incl. 1 table.

Areschoug, J. E. (1874). Observations phycologicae. I. Nova Acta Reg. Soc. Scient. Uppsaliensis VI: 1-26, 4 pls. add.

Astbury, W. T. and R. D. Preston (1940). Structure of the cell wall in some species of Cladophora. Proc. Roy. Soc. (London) B, 129: 55-76, incl. 3 tables, 16 figs; 1 pl. add.

Brand, F. (1901). Ueber einige Verhaltnise des Baues und Wachsthums von Cladophora. Bei. zum Bot. Cent. 10:481-521. (Not seen)

Braun, A. (1855). Algarum unicellularum genera nova et minus cognita. Leipzig.

Collins, F. S. (1918). The green algae of North America. Second supplementary paper. Tufts College Studies. Sci. Ser. 37, 4 (7): 1-106, 3 pls.

Cole, K. (1964). Induced fluorescence in gametophytes of some Laminariales. Can. Jour. Bot. 42:1173-1181, 2 pls. add.

Cronshaw, J., A. Myers and R. D. Preston (1958). A chemical and physical investigation of the cell walls of some marine algae. Biochimica et Biophysica Acta 27:89-103, incl. 6 figs., 9 tables.

Darlington, C. D. and L. LaCour (1960). The handling of chromosomes. 3rd. ed., G. Allen, London. 248 pp., illustrated.

Faberge, A. C. (1945). Snail stomach cytase, a new reagent for plant cytology. Stain Tech. 20:1-4.

Fan, K. C. (1957). Observations on the life history of Codiolum petrocelidis. Phyc. News Bull. 10:32.

Fan, K. C. (1959). Studies on the life histories of marine algae I. Codiolum petrocelidis and Spongomorpha coalita. Bull. Torrey Bot. Club 86:1-12, incl. 41 figs.

Frei, E., and R. D. Preston (1961). Cell wall organization and wall growth in the filamentous green algae Cladophora and Chaetomorpha. 1. The basic structure and its formation. Proc. Roy. Soc. (London) B, 154:70-94, incl. 11 pls.

Frey-Wyssling, A. (1959). Die pflanzlich Zellwand (Springer, Verlag). (Not seen)

Fritsch, F. E. (1942). The algal life-cycle. Ann. Bot. 6:533-563.

Frye, C., and S. M. Zeller (1915) Hormiscia tetraciliata sp. nov. Puget Sound Biol. Sta. Publ. 1:9-13, incl. 1 pl.

Handbook of Chemistry and Physics (1962-1963). 44th ed., Chemical Rubber Publishing Co. 3604 pp.

Hartog, C. den. (1959). The epilithic algal communities occurring along the coast of the Netherlands. Wentia 1:1-241. (Not seen)

Hart, H. T. (1928). Studies on Hormiscia wormskioldii. Puget Sound Biol. Sta. Publ. 5:355-359, incl. 1 pl.

Hirn, K. C. (1900). Monographie und Iconographie der Oedogoniaceen. Helsingfors. 383 pp. (Not seen)

Hoek, Van den, C. (1964). Revision of the European species of Cladophora. E. J. Brill, Leiden. 248 pp., incl. 39 tables, 18 maps; 55 pls. add.

Hollenberg, G. J. (1957). Culture studies of Spongomorpha coalita. Phyc. News Bull. 10:76.

Hollenberg, G. J. (1958). Observations concerning the life cycle of Spongomorpha coalita (Ruprecht) Collins. Madroño 14:249-251, incl. 1 pl.

Iwasaki, H. (1961). The life cycle of Porphyra tenera in vitro. Biol. Bull. 121:173-187, incl. 5 tables, 16 figs.

Jensen, W. A. (1962). Botanical Histochemistry. W. H. Freeman and Co., San Francisco, 408 pp., illustrated.

Jónsson, S. (1957). Sur la reproduction et la sexualité de l'Acrosiphonia spinescens (Kutz.) Kjellm. C. R. Acad. Sci. (Paris) 244:1251-54, incl. 15 figs.

Jónsson, S. (1958). Sur la structure cellulaire et la reproduction de Codiolum petrocelidis Kuck., Algue verte unicellulaire endophyte. C. R. Acad. Sci. (Paris) 247:325-28, incl. 14 figs.

Jónsson, S. (1959a). L'existence de l'alternance heteromorphe de generations entre l'Acrosiphonia spinescens Kjellm. et le Codiolum petrocelidis Kuck. C.R. Acad. Sci. (Paris) 248:1565-67. incl. 1 fig.

Jónsson, S. (1959b). Le cycle de développement du Spongomorpha lanosa (Roth) Kutz. et la nouvelle famille des Acrosiphoniacées. C.R. Acad. Sci. (Paris) 248:1565-67, incl. 1 fig.

Jónsson, S. (1960) Nouveau type de cytodiérese de la cénocytique observé chez une Algue verte filamenteuse. C.R. Acad. Sci. (Paris) 251:2390-2392, incl. 4 figs.

Jónsson, S. (1962). Thèsis. Recherches sur des Cladophoracées marines (structure, reproduction, cycles comparés, consequences systématiques). Ann. Sc. Nat. Bot., 12e serie, 3:25-230, incl. 4 tables, 48 figs; 16 pls. add.

Jónsson, S. (1963). Sur quelques variations du cycle de dévelopment dans la famille des Acrosiphoniacées. C.R. Acad. Sci. (Paris) 256:5187-5189.

Jónsson, S. (1964a). Nouveau type de parthénogenèse haploïds chez les Algues: la parthénogenèse plasmogamique. C.R. Acad. Sci. (Paris) 258:2145-2148, incl. 1 fig.

Jónsson, S. (1964b). Existence d'une caryogamie facultative chez l'Acrosiphonia spinescens (Kutz.). C.R. Acad. Sc. (Paris) 258:6207-6209.

Jorde, I. (1933). Untersuchungen über den Lebenszyklus von Urospora Aresch. und Codiolum A. Braun. Nyt. Mag. Naturvid. 73:1-19, incl. 5 figs., 1 pl.

Juller, E. (1937). Der Generations und Phasenwechsel bei Stigeoclonium subspinosum. Arch. Protist. 89:53-93. (Not seen)

Kornmann, P. (1961a). Über Spongomorpha lanosa und ihre Sporophytenformen. Helgol. Wiss. Meeresunters. 7:195-205, incl. 6 figs.

Kornmann, P. (1961b). Die Entwicklung von Codiolum gregarium A. Braun, Helgol. Wiss. Meeresunters. 7:252-259, incl. 5 figs.

Kornmann, P. (1961c). Über Codiolum und Urospora. Helgol. Wiss. Meeresunters. 8:42-57, incl. 9 figs.

Kornmann, P. (1962). Eine Revision der Gattung Acrosiphonia. Helgol. Wiss. Meeresunters. 8:219-242, incl. 11 figs.

Kornmann, P. (1963). Die Ulotrichales, neu geordnet auf der Grundlage entwicklungsgeschichtlicher Befunde. Phycologia 3:60-68, incl. 1 fig.

Kornmann, P. (1964a). Die Ulothrix-Arten von Helgoland.I. Helgol. Wiss. Meeresunters. 11:27-38 incl. 8 figs.

Kornmann, P. (1964b). Uber Monostroma bullosum (Roth) Thuret und M. oxyspermum (Kutz.) Doty. Helgol. Wiss. Meeresunters. 11:13-21, incl. 5 figs.

Kuckuck, P. (1894). Bemerkungen zur marinen Algenvegetation von Helgoland, Wiss. Meeresunters. Biolog. Anstalt. Helgol. N.F., 1:225-263, incl. 29 figs.

Ledbetter, M. C. and K. R. Porter (1963). A "microtubule" in plant cell fine structure. Jour. Cell Biol. 19:239-250, incl. 8 figs.

Manton, I. (1952). The fine structure of plant cilia. Symp. Soc. Exp. Biol. 6:306-319, incl. 11 pls.

Manton, I., Clarke, B. and Greenwood, A. D. (1955). Observations with the electronmicroscope on biciliate and quadriciliate zoospores in green algae. Jour. Exp. Bot. 6:126-128, 10 figs. add.

Manton, I. (1964) Observations on the fine structure of the zoospore and young germling of Stigeoclonium. Jour. Exp. Bot. 15:399-411, incl. 41 figs.

McLung, C. E. (1937). Handbook of Microscopical Technique. 2nd edit. Paul B. Hoeber Inc., New York, 698 pp., illustrated.

Moewus, F. (1938). Die sexualität und der Generationswechsel der Ulvaceen und Untersuchungen über die Parthenogenese der Gameten. Arch. Protistenk. 91:357-441. (Not seen)

Newcomer, E. H. (1953). A new cytological and histological fluid. Science 118:161.

Nicolai, E., and R. D. Preston (1952). Cell-wall studies in the Chlorophyceae. I. A general survey of submicroscopic structure in filamentous species. Proc. Roy. Soc. (London) B, 140: 244-274, incl. 4 tables, 21 figs.

Printz, H. (1926). Die Algenvegetation des Trondhjemsfjordes. Norske Vidensk.-Akad. Oslo, Skrifter, I. Mat.-naturw. Klasse, No. 5, 273 pp., incl. 8 tables, 29 figs., 10 pls., 1 map.

Printz, H. (1932). Observations on the structure and reproduction in Urospora Aresch. Nyt. Mag. Naturvidensk. 70:273-287, 2 pls. add.

Sarma, Y. S. R. K. (1963). Contribution to the karyology of the Ulotrichales Phycologia 2:173-183, incl. 17 figs.

Scagel, R. F. (1957). An annotated list of the marine algae of British Columbia and Northern Washington. (Including keys to genera) Nat. Museum Can. Bull. No. 150, Biol. Ser. No. 52, 289 pp.

Setchell, W. A., and N. L. Gardner (1920). The marine algae of the Pacific coast of North America. Part 2, Chlorophyceae. Univ. Calif. Publ. Bot. 8:139-374, plates 9 - 33 add.

Smith, G. M. (1938). Nuclear phases and alternation of generations in the Chlorophyceae. Bot. Rev. 4:132-139.

Smith, G. M. (1944). Marine algae of the Monterey Peninsula, California. Stanford Univ. Press. ix 622 pp., incl. 98 pls.

Smith, G. M. (1946). On the structure and reproduction of Spongomorpha coalita (Rupr.) Collins. Jour. of the Indian Bot. Soc. Iyengar commemoration volume Pp. 201-208, incl. 1 table, 5 figs.

Starr, R. C. (1956). Culture collection of algae at Indiana University. Lloydia 19:129-149.

Taylor, W. R. (1957). Marine algae of the Northeastern Coast of North America. 2nd ed. Univ. Michigan Press. Ann Arbor. 509 pp. incl. 60 pls.

Tiffany, L. H. (1924). A physiological study of growth and reproduction among certain green algae. Ohio Jour. Sci. 24:65-98, incl. 6 figs.

Wille, N. (1900). Die Zellkerne bei Acrosiphonia (J. Ag.) Kjellm. Bot. Centralbl. 81:238-239. (Not seen).

Wurdack, M. E. (1923). Chemical composition of the walls of certain algae. Ohio Jour. Sci. 23:181-191, incl. 6 figs.

Zimmerman, W. (1925). Helgolander Meeresalgen I-IV. Beiträge zur Morphologie, Physiologie und Ökologie der Algen. Wiss. Meeresunters, N.F. Abt. Helgoland 16:1-25, 1 pl. add.

TABLE 1

COLLECTION SITES: CLONES ESTABLISHED AND FIELD TRIP DATA (June, 1963)

M - Mid-tide
H - High mid-tide
L - Low tide

NO.	COLLECTION SITES	POSITION LAT. N. °	′	LONG. W. °	′	DESCRIPTION OF AREA	TIDAL LEVEL	NO. OF UROSPORA CLONES SET UP	June 1963 UROSPORA SEXUAL	June 1963 CODIOLUM PRESENT
	VANCOUVER ISLAND, B.C.									
2	Sidney	48	39	123	23	Foot of Beacon Ave., 100 yds. south	M	1	S	-
3	Oak Bay	48	25	123	18	Below the Towers Apts, 1270 Beach Drive	M	1♀, 1♂	S	C
4	Ogden Pt. breakwater*	48	24	123	23	South side of breakwater and adjacent beach	M	1♀, 1♂	S	C
5	Esquimalt	48	25	123	26	South of Yarrows Shipyard, 200 ft. (Duntze Head)	M	1	S	
8	Long Beach	48	59	125	37	South of Long Beach Store, 100 ft.	H M	1 1♀, 1♂	S	
9	Port Renfrew	48	31	124	27	Southwest area of Botany Beach	H M L	1 1♀ 1	S	-
10	Point No Point	48	23	123	55	About 125 yds. northeast of the point	H M L	1 1♀, 1♂ 1,6 !!	S	C
11	Kelsey Bay	50	23	125	58	Below Kelsey Bay Dock and north 100 yds.	L	10	-	-
12	Campbell R.	50	01	125	13	South side of Discovery Crescent jetty	M	1	?	-
13	Qualicum Beach	49	21	124	27	Areas with boulder outcrops	M	1	?	-
14	Departure Bay	49	12	123	57	Southeast of ferry slip, 200 ft.	M	1	-	
	SAN JUAN IS.,WASH.,U.S.A.									
15	Deadman's Bay	48	30	123	08	A small bay 700 yds. south of Deadman's Bay	H M L	5 1♀, 1♂ 5, 2,8!	S	C
16	Friday Harbor	48	33	123	00	Below the new laboratory	M	1	S	C
	FIDALGO IS.,WASH., U.S.A.									
17	Anacortes	48	30	122	41	East of Shannon Point to the Gulf Island Ferry Terminal	M	1♀, 1♂	S	C
	VANCOUVER, B.C.									
18	Tsawwassen Ferry jetty	49	00	123	05	On both sides of the jetty past the ferry depot	H M L	5 3♀, 4♂ 10,10,10	S	C
19	Pt. Atkinson	49	20	123	16	Northwest of lighthouse, 50 - 100 yds.	M	1	S	-
	GALIANO ISLAND, B.C.									
20	Porlier Pass	49	01	123	35	Below the Virago Point lighthouse	M			-

* Also referred to as the Victoria Harbour breakwater.

! Urospora speciosa, !! Urospora vancouveriana, ♀♂Urospora wormskioldii

TABLE 2

FILAMENT GROWTH IN UROSPORA WORMSKIOLDII

SOLUTION NO.	SEA WATER	KNO_3	KH_2PO_4	FeEDTA	TRIS	Na-glycerol phosphate	SOIL WATER	$NaNO_3$	Na_2HPO_4	BIOTIN	THIAMINE	B_{12}	REPLICATES	42 DAYS GROWTH Relative Density* Approx. Filament Length (cm.)
	ml.	mg.	mg.	mg.	mg.	mg.	ml.	mg.	g.	µg.	µg.	µg.		1 2 3
1	1000	(SEAWATER)											1	
													2	
													3	
													4	
2	1000	(ERDSCHREIBER)					50	100	20				1	
													2	
													3	
													4	
3	1000	72.2	8.8	0.5	500	10.5				6			1	
		(SWII)											2	
													3	
													4	
4	1000	72.2	8.8	0.5	500	10.5				6	10	0.02	1	
		(SWII + BIOTIN + THIAMINE + B12)											2	
													3	
													4	
5	1000	72.2	8.8	0.5	500								1	
		(SWI)											2	
													3	
													4	

* Bar width represents approximate fraction of flask-bottom covered with filaments.

TABLE 3

GERMINATION OF SINGLE ZOOSPORES AND CARRY-OVER OF SEXUALITY IN UROSPORA WORMSKIOLDII FEMALE CLONE F11

SOLUTION	REPLICATE NO.	GERMINATION and GROWTH of ZOOSPORES: June 20, 63	July 2, 63	Aug. 6, 63	Sept. 30, 63	Nov. 14, 63	Dec. 7, 63	SEXUALITY ON SUBCULTURE in UR1 at 10°C. DEC. 11-31		JAN. 1-20	
								C	S	C	S
DAY		0	13	48	103	133	148	C	S	C	S
1	1	CULTURES STARTED	✓	~500 (9 celled)	No Growth Visible, Fil. Microscopic	Filaments look Dead Added 1 ml sol.5 to each	■	↑		↑	↑ ↘	↑	↑ ↘
	2		✓	~100 "			■	↑		↑	↑ ↘	↑	↑ ↘
	3		✓	~ 40 "			■	↑	↘	↑		↑	↑ ↘
	4												
	5		✓	1 only (5 mm)			■	↑		↑	↑ ↘	↑	↑ ↘
4	1		✓	>100 1-2 cm.	■	↑	■	↑	↘			↑	↑ ↘
	2												
	3		✓	>100 "	■	↑	■	↑		↑	↑ ↘	↑	↑ ↘
	4		✓	>100 "	■	↑	■	↑		↑	↑ ↘	↑	↑ ↘
	5		✓	>100 "	■	↑	■	↑		↑	↑ ↘	↑	↑ ↘
	6		✓	>100 "	■	↑	■	↑		↑	↑ ↘	↑	↑ ↘
	7												
	8												
	9		✓	>100 "	■	↑	■	↑		↑	↑ ↘	↑	↑ ↘
	10		✓	>100 "	■	↑	■	↑	↘	↑	↑ ↘	↑	↑ ↘
5	1												
	2		✓	>100 "	■	↑	■	↑		↑	↑ ↘	↑	↑ ↘
	3		✓	>100 "	■	↑	■	↑					
	4		✓	>100 "	■	↑ ↘	■	↑	↘	↑	↑ ↘	↑	↑ ↘
	5		✓	>100 "	■	↑	■	↑		↑		↑	↑ ↘

✓ Germination occurred ↘ Sexual ↑ Codiolum

■ Abundant filament growth, filaments 2-3 cm long.

Sol. 1 = Seawater

Sol. 4 = SW1 + Biotin, Thiamine, B12

Sol. 5 = SW1

* UR1 = SW1 minus TRIS; supplemented with 6 µg/l Biotin, 10 µg/l Thiamine, 0.02 µg/l B12

TABLE 4

GERMINATION OF SINGLE ZOOSPORES OF UROSPORA WORMSKIOLDII MALE CLONE M2A

COMPOSITION OF MEDIA USED											
COMPOSITION		5-1	5-2	5-3	5-4	5-6	5-7	5-8	5-9	5-10	5-11
SEA WATER (32.7‰)	ml.	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
KNO_3	mg.	72.2	72.2	72.2	72.2			72.2	72.2	72.2	72.2
KH_2PO_4	mg.	8.8	8.8	8.8	8.8			8.8	8.8	8.8	8.8
$NaNO_3$	mg.	-				100	100				
Na_2HPO_4	mg.	-				20	20				
Fe EDTA	mg.	0.5	0.5	0.5	0.5		0.5				
TRIS	mg.	1000	500	250	0			1000	500	250	0
SOIL WATER	ml.	-				50	50				
Growth after 50 days culture	REPLICATES 1	▌	▌	▌	■	▌	▌				
	2	▌	▌	▌	■	▌	▌				
	3	▌	▌	■	■	▌	■				
	4	▌	▌	■	■	▌	▌				

▌ Sparse growth, filaments under 2 mm long.

■ Abundant growth, filaments 2-3 cm long.

TABLE 5

EFFECT OF TEMPERATURE ON FERTILITY OF NATURAL CODIOLUM

Sept. 22 - Oct. 29, 1963 0/0 FERTILITY													
T°C		0 DAY	4	8	10	14	16	28	30	32	34	36	38
5°	D	0		0		40		100					
	F	0		0		40		100					
10°	D	0		0		1		100					
	F	0		2		2		100					
15°	D	0		0		0		0	DISCONTINUED				
	F	0		.5		0		10	Returned to 10°C				0 2
20°	D	0		0		0		0	DISCONTINUED				
	F	0		0		0		0	Returned to 10°C				0 0

D - Codiolum gregarium from a rock at Deadman's Bay.
F - C. gregarium from a log at Friday Harbor

TABLE 6

EFFECT OF THERMOPERIOD ON FERTILITY OF NATURAL CODIOLUM

Aug. 18 - Sept. 19 1963	0/0 FERTILITY																	
	0	1	DAY	4	5	6	7	8	9	10	11	12	13	17	18	19		
CONTROL	0											60				97	13 in nuclear division 15 fertile 51 not fertile	
TEST	0	Thermoperiod										0				19		

Thermoperiod - 8 hrs at 15 - 18°C coincident with an 8 hr photoperiod.

Basal temperature - 10°C.

TABLE 7

EFFECT OF COLD SHOCK ON FERTILITY OF CULTURAL CODIOLUM

Codiolum Isolate No.	0/0 FERTILITY														
	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16
8L♀						%	0		18.8	8.1		34.6			61.1
						TCE			32	57		65			54
UrX1						%			0	0		0			0
						TCE	0		100	100		84			86
8L♀								%	0	12		9.4			34.5
		-2° C, Dark						TCE		52		85			55
UrX1								%	0	0		0			0
								TCE	100	100		106			107
8L♀	CONTROL NOT FERTILE														
UrX1	CONTROL NOT FERTILE														

TCE - Total cells examined.

TABLE 8

EFFECT OF TEMPERATURE ON FERTILITY OF NATURAL CODIOLUM

Origin / Temp.	Zygotes (8L x 18-3)	M2A ♂	F11 ♀	URX1	F3 ♀
5°C	-	-	-	-	-
10°C	one cell	-	-	-	3%
15°C	-	-	-	-	3%
20°C					-

% - Percentage of Codiolum cells fertile after 60 days growth.

TABLE 9

EFFECT OF TEMPERATURE ON SEXUALITY IN UROSPORA WORMSKIOLDII

Temp. / Clone number	20°C	15°C	10°C	5°C
F11 ♀	S	S	-	-
M2A ♂	NG	S	-	-
URX1	NG	-	-	-

S - Sexual, NG - No growth.

Key to symbols in Figures 1 - 40

c	crosswall
cb	chromatic body
ch	chloroplast
e	eyespot
f	zoospore ridge fibril
fl	flagellum
g	gametangium
HHW	higher high water
l	pectic lamella
MHHW	mean higher high water
MLLW	mean lower low water
n	nucleus
nu	nucleolus
o	operculum
p	discharge pore
py	pyrenoid
R	reduction division
s	septa-like structure
sh	sheath
z	zoosporangium

FIGURE 1

LIFE CYCLE OF UROSPORA MIRABILIS ARESCH.

(After Jorde, 1933)

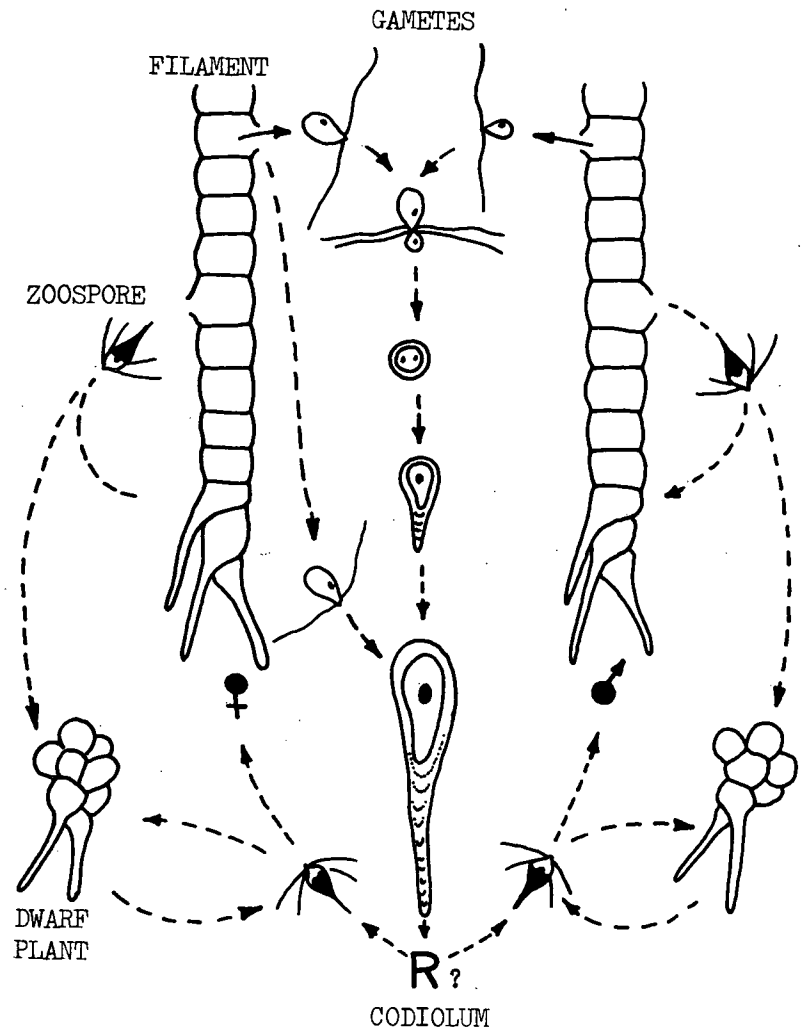


FIGURE 2

LIFE CYCLES IN UROSPORA AND ULOTHRIX SPECIES

(After Kornmann, 1963)

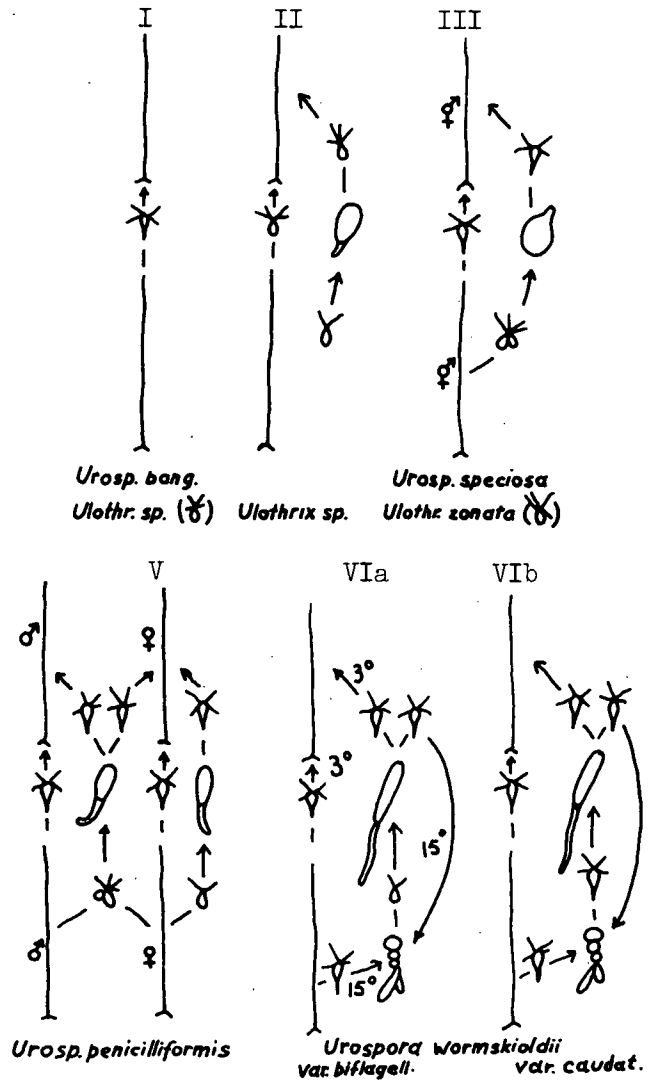


FIGURE 3

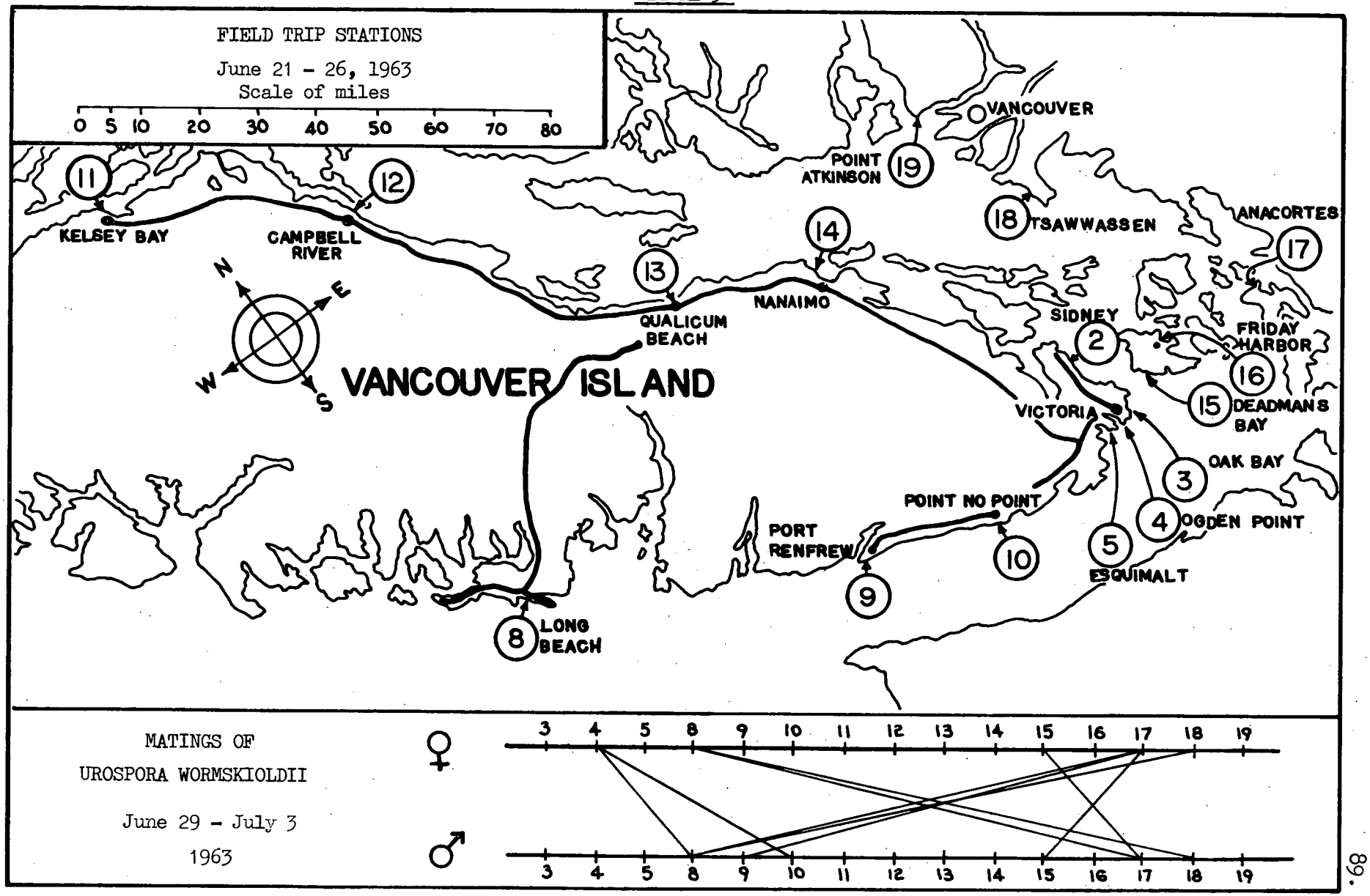

FIELD TRIP STATIONS
June 21 - 26, 1963
Scale of miles
0 5 10 20 30 40 50 60 70 80
VANCOUVER ISLAND
N
E
S
W
11 KELSEY BAY
12 CAMPBELL RIVER
13 QUALICUM BEACH
14 NANAIMO
19 POINT ATKINSON
VANCOUVER
18 TSAWWASSEN
ANACORTES
17
SIDNEY
2
FRIDAY HARBOR
16
15 DEADMAN'S BAY
VICTORIA
3 OAK BAY
4 OGDEN POINT
5 ESQUIMALT
POINT NO POINT
10
PORT RENFREW
9
8 LONG BEACH
MATINGS OF
UROSPORA WORMSKIOLDII
June 29 - July 3
1963
♀
♂
3 4 5 8 9 10 11 12 13 14 15 16 17 18 19

FIGURE 4

A. Tsawwassen Ferry Jetty, April 1, 1964 NW side, looking NE.
(1) Upper limit of Codiolum gregarium (13 ft. level).

B. Close up of rectangular area in (A).
(1) Large form of Urospora wormskioldii collected here April - May, 1963 - 1964 at low tide (3 ft.) Refer to Fig. 24 for plant form.

(2) U. wormskioldii most abundant on rocks in the mid-tide (5 - 8 ft.) but extending down to (1), (3 ft.) and up to (3), (11 ft.)

C. Ogden Point Breakwater, June 21, 1963.
(1) Upper and lower limit of C. gregarium, (9.5 - 11.5 ft), third tier from the top.

(2,3) U. wormskioldii in the mid-tide, (4 - 9 ft). Water level 4 ft.

D. Oak Bay, June 21, 1963.
(1) Position at which C. pusillum appeared in the fall.

(2) U. wormskioldii in the mid-tide region.

E. Rocks obtained at (1) in (D) with a dense covering of C. pusillum, October 9, 1963.

F. Point No Point, August 8, 1963 looking NE.
(1) U. vancouveriana, on rocks in the low tide area (3 - 0 ft). Water level 2.5 ft. U. wormskioldii from the water line up to the 9 ft. level.

G. Deadman's Bay, August, 1961.
(1) Area where large forms of Urospora were collected in the spring of 1961, 1963 and 1964. (Refer to Fig. 24 for plant form) and where U. speciosa was collected April 1, 1964. Tidal level at (1) is 1 - 2 ft.

(2) Rock on which a small patch (4 sq. in.) of fertile C. gregarium was found, January 1961. Tidal level at (2) is 9 - 10 ft.

(3) Rock referred to in Fig. 29G having C. gregarium on the upper surface and U. wormskioldii on the lower.

FIGURE 4

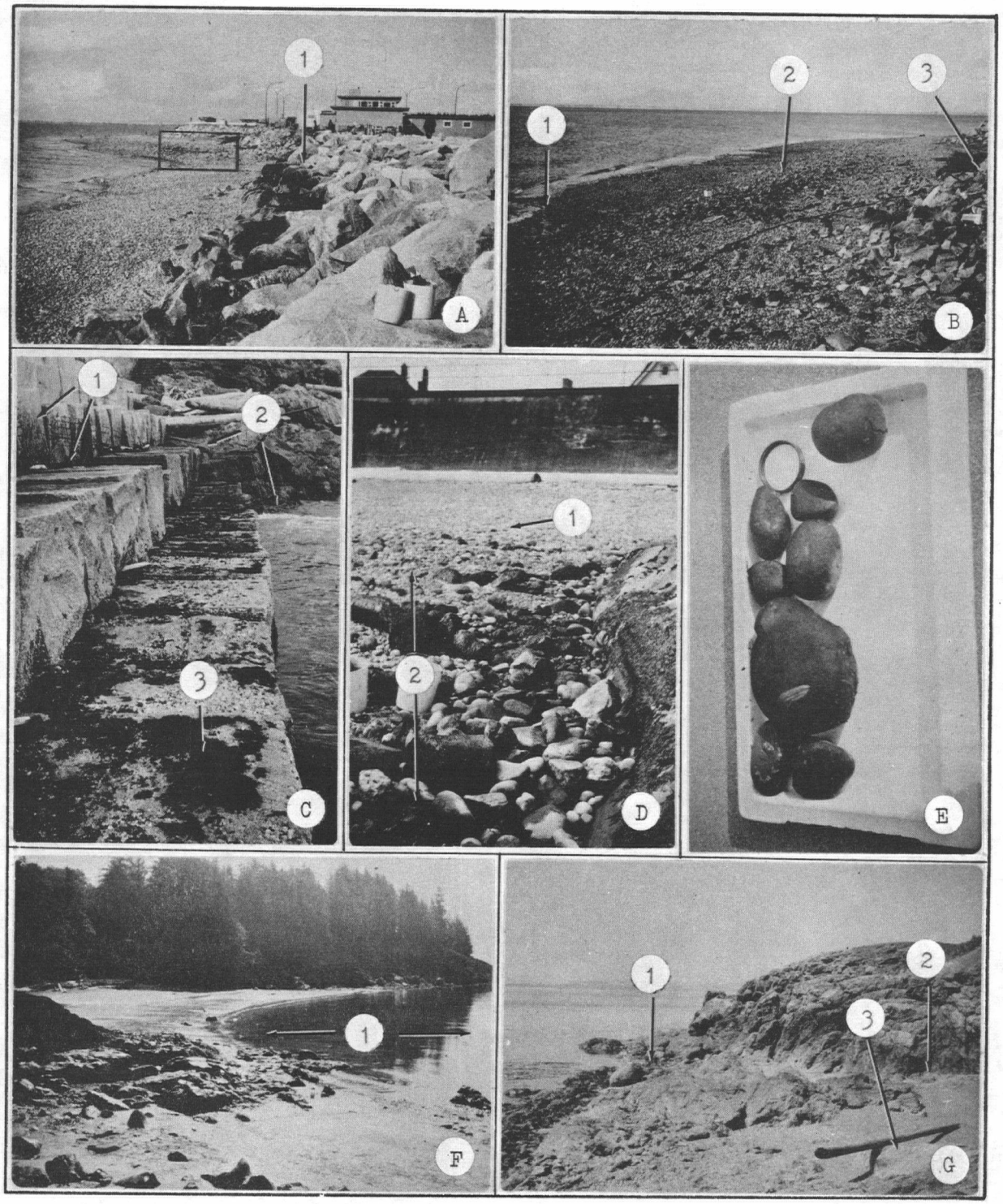


FIGURE 5

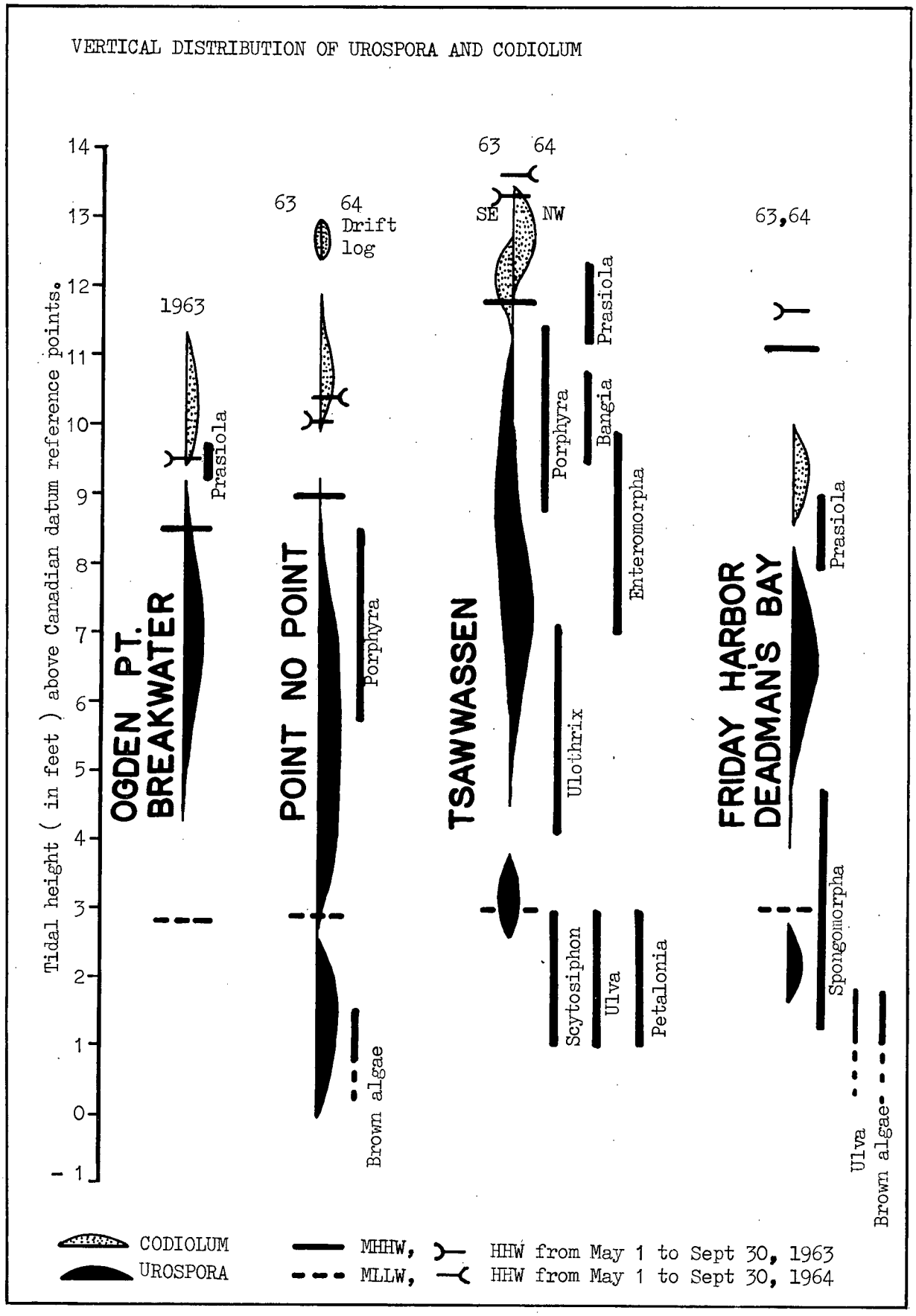

VERTICAL DISTRIBUTION OF UROSPORA AND CODIOLUM
Tidal height (in feet) above Canadian datum reference points.
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
- 1
OGDEN PT. BREAKWATER
1963
Prasiola
POINT NO POINT
63
64
Drift log
Porphyra
Brown algae
TSAWWASSEN
63
64
SE
NW
Porphyra
Bangia
Prasiola
Enteromorpha
Ulothrix
Scytosiphon
Ulva
Petalonia
FRIDAY HARBOR
DEADMAN'S BAY
63,64
Prasiola
Spongomorpha
Ulva
Brown algae
CODIOLUM
UROSPORA
MHHW,
MLLW,
HHW from May 1 to Sept 30, 1963
HHW from May 1 to Sept 30, 1964

FIGURE 6

CODIOLUM - VARIATION IN ABUNDANCE AND MORPHOLOGY

STATIONS NO	LOCALITY	Approx. Tide Level	SEASONAL VARIATION IN ABUNDANCE	DESCRIPTION	TYPE: Codiolum gregarium	TYPE: Codiolum pusillum
			A O D F A J			
15	DEADMAN'S BAY	9-10	60 61	One patch, 4 sq.in., on one rock.	●	
		9-10	62 63	One patch, 4 sq.ft., on one rock.	◆	
16	FRIDAY HARBOR	9-10	63 64	On east and west side of sunken log in a 6 in. band, 10 ft. long	●	
18	TSAWWASSEN JETTY	11-12	62 63	Patches, 1-2 sq.ft., on many rocks on the SW and SE side.	●	
		12-13	63 64	Rocks on the SW side only	●	
		13	63 64	One streak, 1 ft. x 6 in. on a drift log.	●	
4	OGDEN POINT BREAKWATER	9-11	62 63	Lower 2 ft. on the vertical surface of the third tier blocks.	◆	
			63 64	Horizontal surface of fourth tier blocks.	●	
10	POINT NO POINT	10-12	62 63	Few cells from several rocks.	●	
		12.5	62 63	Streaks, ½ x 12 in. on NW side of drift log.	●	
			63 64		●	
3	OAK BAY	10	62 63 *	On rocks, 4-12 in. diameter, upper surfaces completely covered	◆	

—— Scarce - A few cells found from several rock scrapings.

—— Common - Scattered patches, about 2 sq. in. in diameter, cells widely separate.

Abundant - Patches close together.

Very abundant - Large surfaces covered, 2-6 sq. ft., cells closely appressed.

◆ Relative amounts of form variants.

* Worn away by rock abrasion during October gales.

FIGURE 7

LIFE CYCLE OF UROSPORA WORMSKIOLDII (MERTENS) ROSENVINGE

(TYPE I)

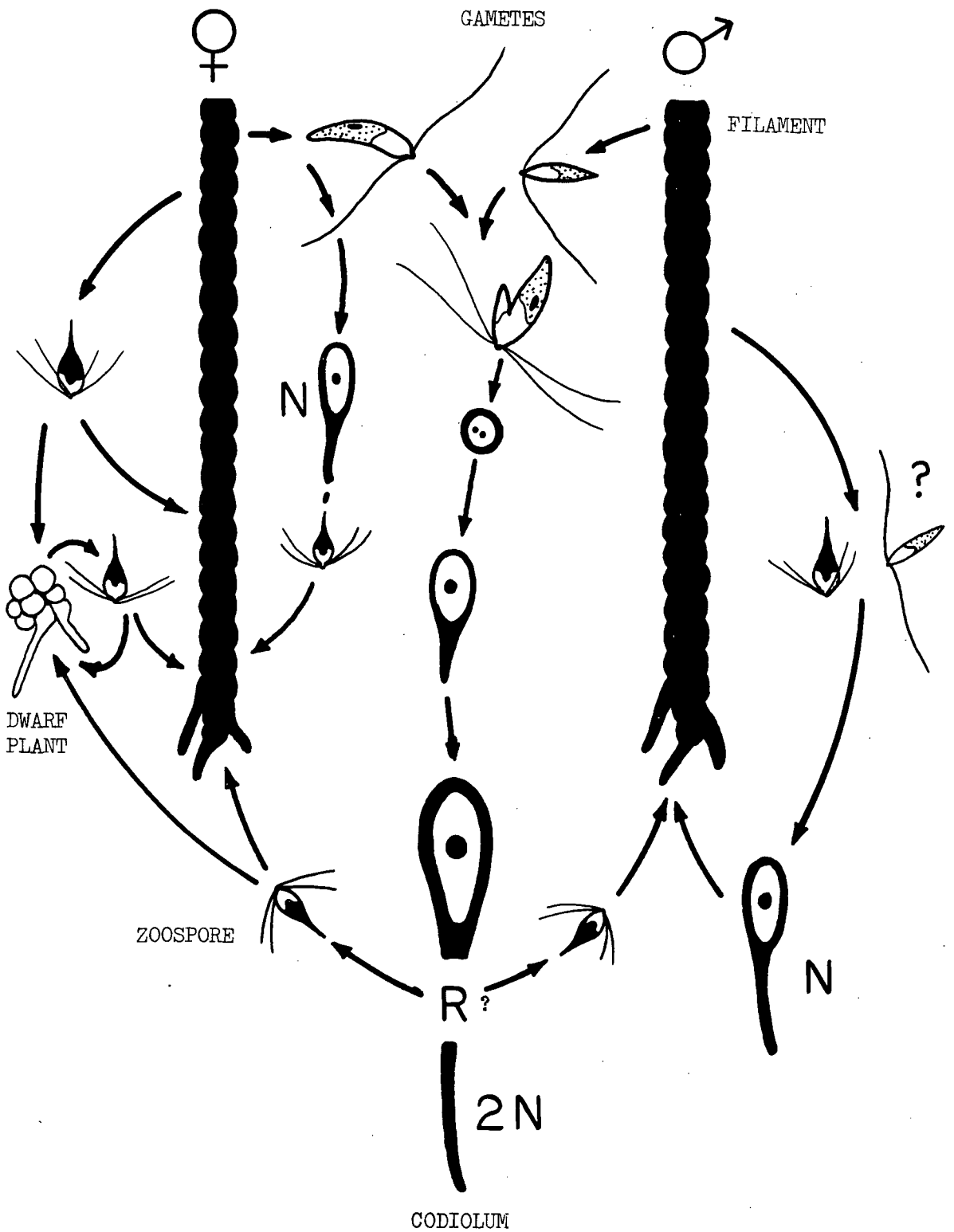


FIGURE 8

Urospora wormskioldii

(A - D, F - J, field. E, culture)

A - C Sections of filaments from Kelsey Bay, June 24, 1963.
A. Basal cell with coarsely perforate chloroplast withdrawn from the end walls. x 800.

B. Basal cells of another filament with the chloroplast extending to the end walls. x 500.

C. Cells further up the same filament as (B) with the chloroplast perforations becoming finer. IKI. x 400.

D. Filament showing abnormal cell division. Two cells are wound about each other resulting from oblique wall formation. x 500. Insert, x 50.

E. Test tube culture of male clone M2A showing a ring of Codiolum (cr) at the original air-water interface.

F. Cell showing unconnected ribbon-like chloroplasts. x 500.

G - J Developmental stages in zoospore formation.
G. Finely reticulate chloroplast with conspicuous pyrenoids. IKI. x 500.

H. Chloroplast becoming disorganized, pyrenoids inconspicuous. x 500.

I. Zoospore delimitation. x 500.

J. Beginning of zoospore elongation. x 500.

Scale length, 50 μ

FIGURE 8

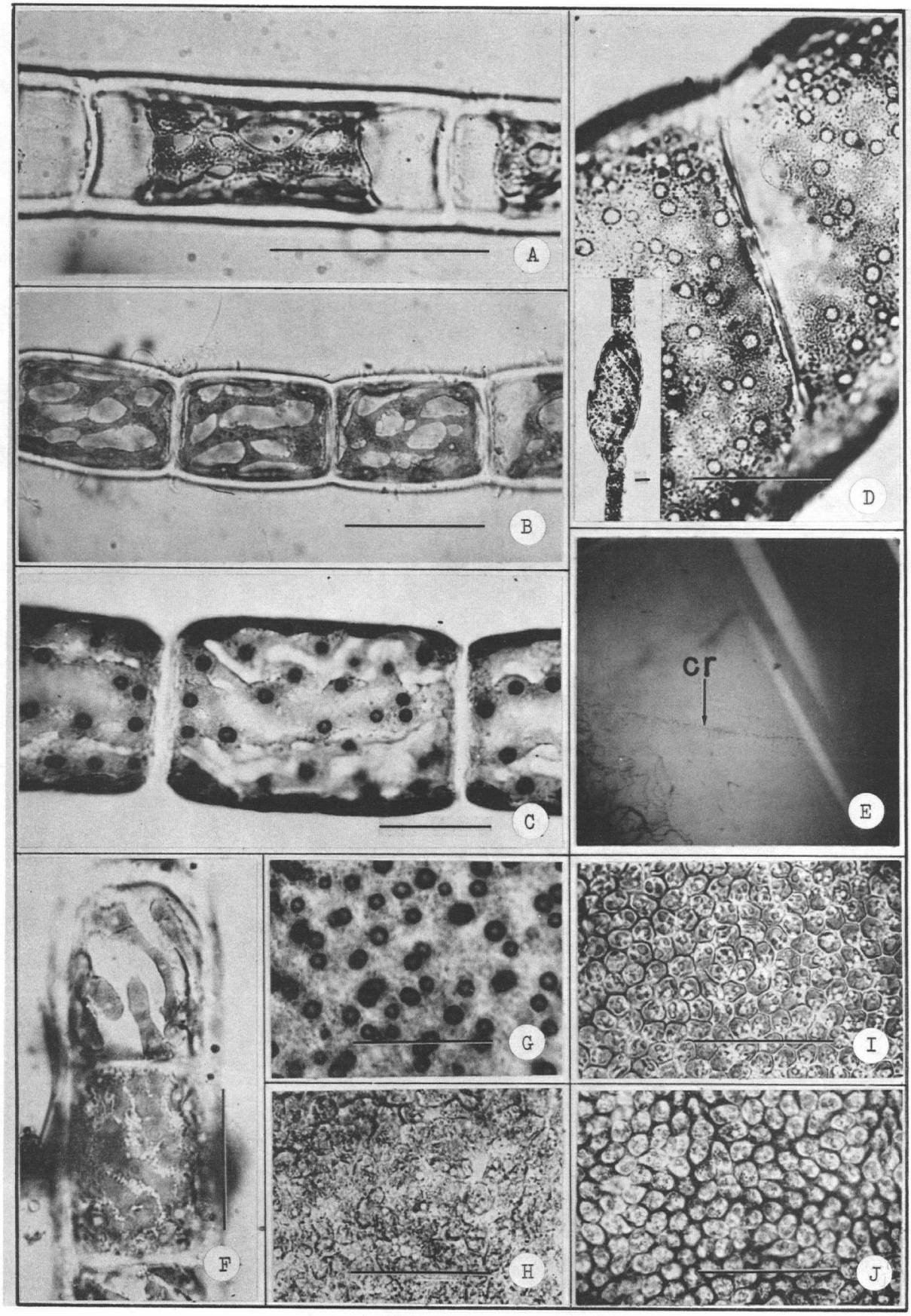


FIGURE 9

Urospora wormskioldii

(A, B, D, E, G, field. C, F, H, I, culture)

A, B. Formation of discharge pores (p) in sporangia. Safranin. x 1000.

C. Ten-day-old germlings from zoospores. Acetocarmine. x 400.

D, E. Zoospore release in vesicles. x 400.

F. Dwarf plants after six months in 10 °/oo salinity seawater. Many rhizoids formed. IKI, eosine. x 500.

G. Wall structure in empty sporangia. x 500.

H, I. Dwarf plants after six months in 50 °/oo salinity seawater. Many large thick-walled cells produced. Rhizoids absent. H, x 400. I, x 80.

Scale length, 50 µ

FIGURE 9

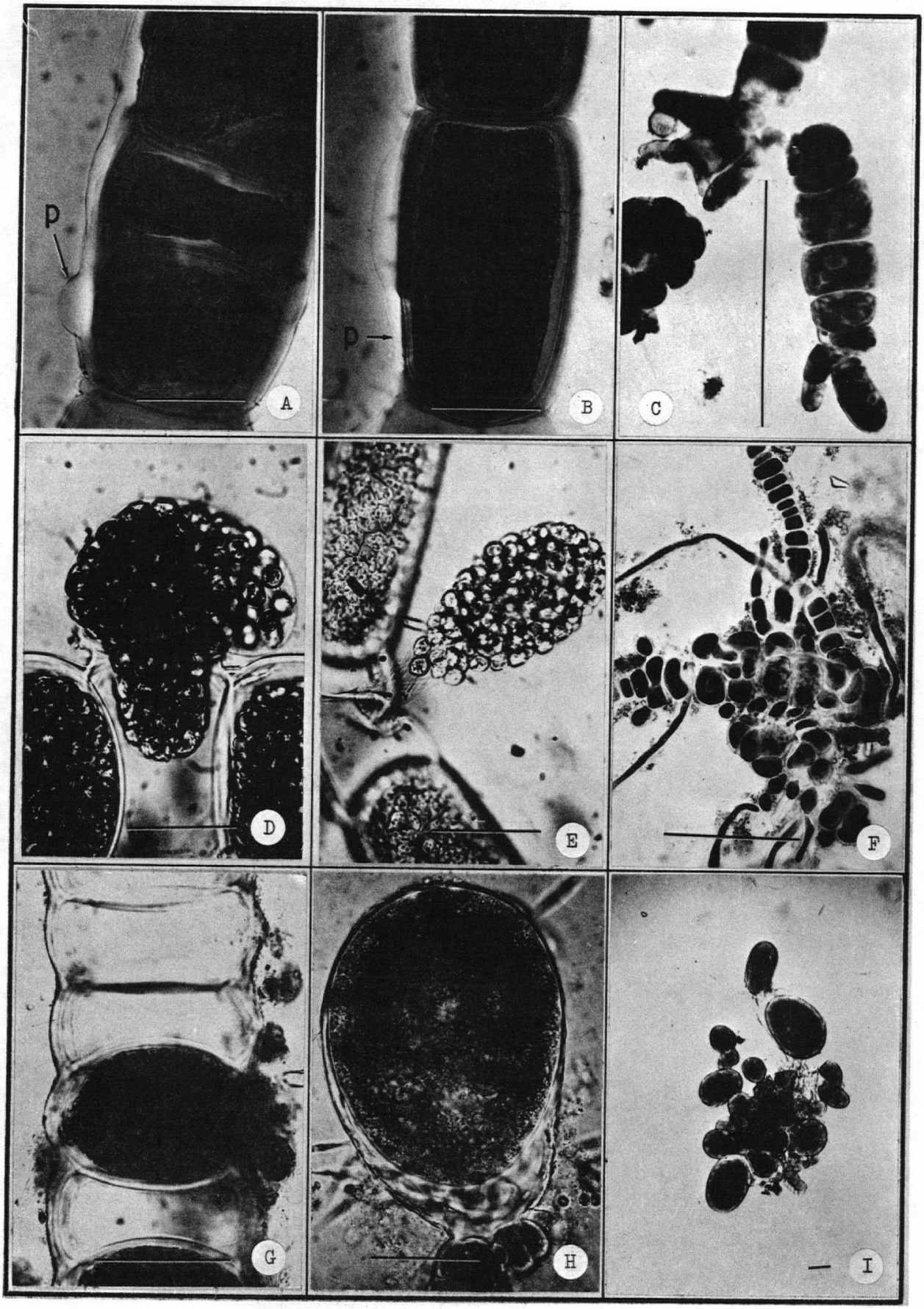


FIGURE 10

Urospora wormskioldii

(Living field material from Deadman's Bay, 1961)

A - C. Sections of one filament showing an increase in width apically. Rhizoids intramatrical. Note fourth cell from top growing through fifth cell. x 100.

D. Sections of several filaments showing cells in different stages of development. x 50.

E. Chloroplast of mature cell showing pyrenoids and perforate nature of the chloroplast. x 900.

F. Surface of fertile sporangium showing star-like clusters of zoospores. x 500.

G. Photograph of field plants *in situ* in the mid-tide region. x 2/3.

H. Freshly liberated zoospores with four flagella and long finely-drawn-out tails. x 500.

Scale length, 50 /u

FIGURE 10

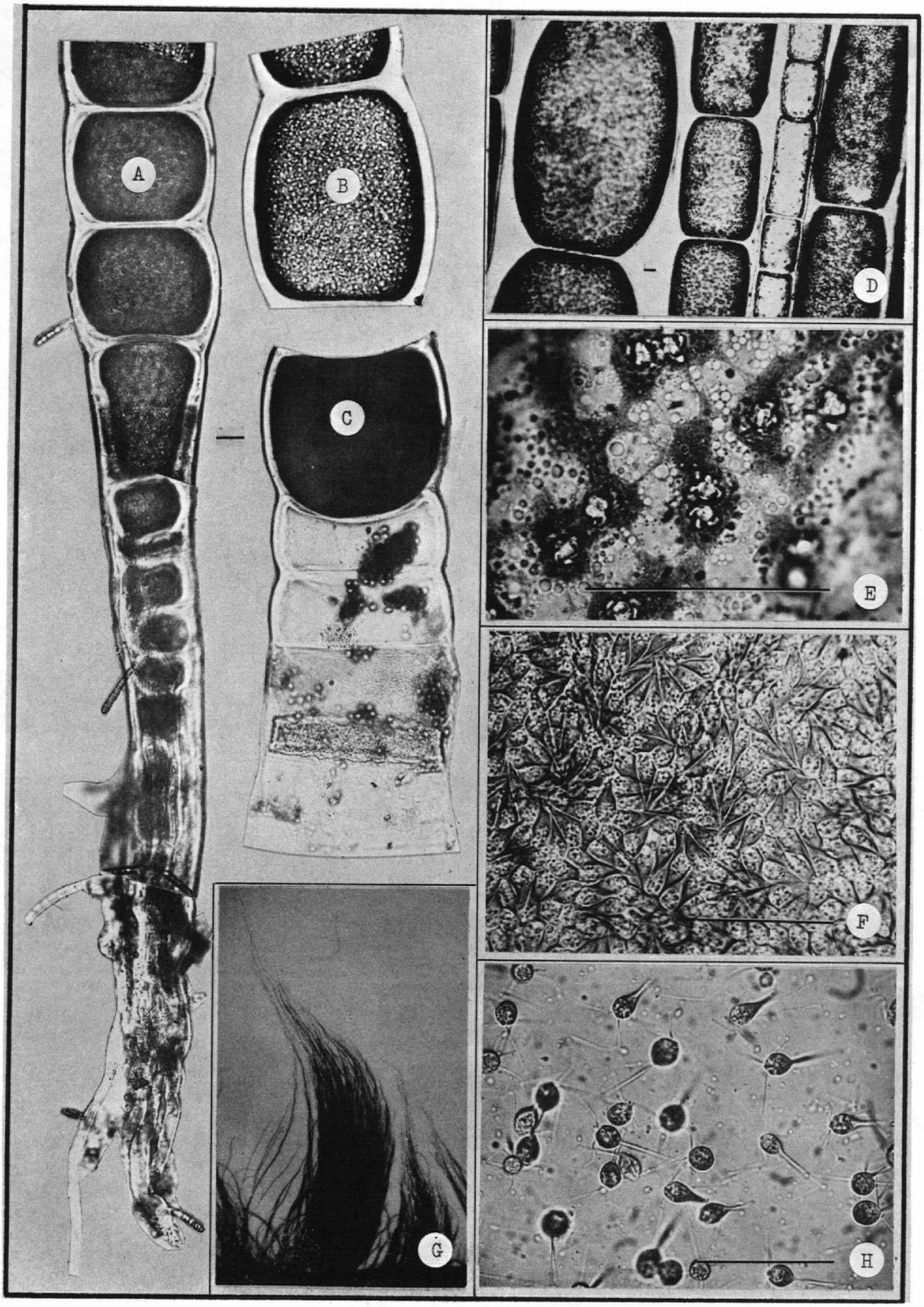


FIGURE 11

Urospora wormskioldii

(A - E, G - I, culture. F, field. Living material)

A. Sections of filament from base to tip showing extramatrical rhizoids and decreasing filament width. x 100.

B. Cell of filament in (A) with finely perforate chloroplast. x 500.

C. Cells of filament in (A) with banded chloroplast. x 500.

D. Male clone M2A, fertile zoosporangium (z) and fertile gametangium (g). x 900.

E. Zoospore squashed showing the four "ridge" fibrils (f) and four flagella (fl). x 2000.

F. End wall view of mature cell of field filament showing chloroplast absent at both end walls. x 100.

G. Coarsely-perforate chloroplast showing prominent nuclei (n) and nucleoli (nu). x 1000.

H. Fertile sporangium with zoospore arrangement reflecting a previous coarsely-reticulate chloroplast. x 1000.

I. Cultural zoospores with short tails. x 500.

J. Field zoospore. Acetocarmine. Phase. x 2000.

Scale length A - D, F - I, 50 /u; E, 25 /u; J, 20 /u

FIGURE 11

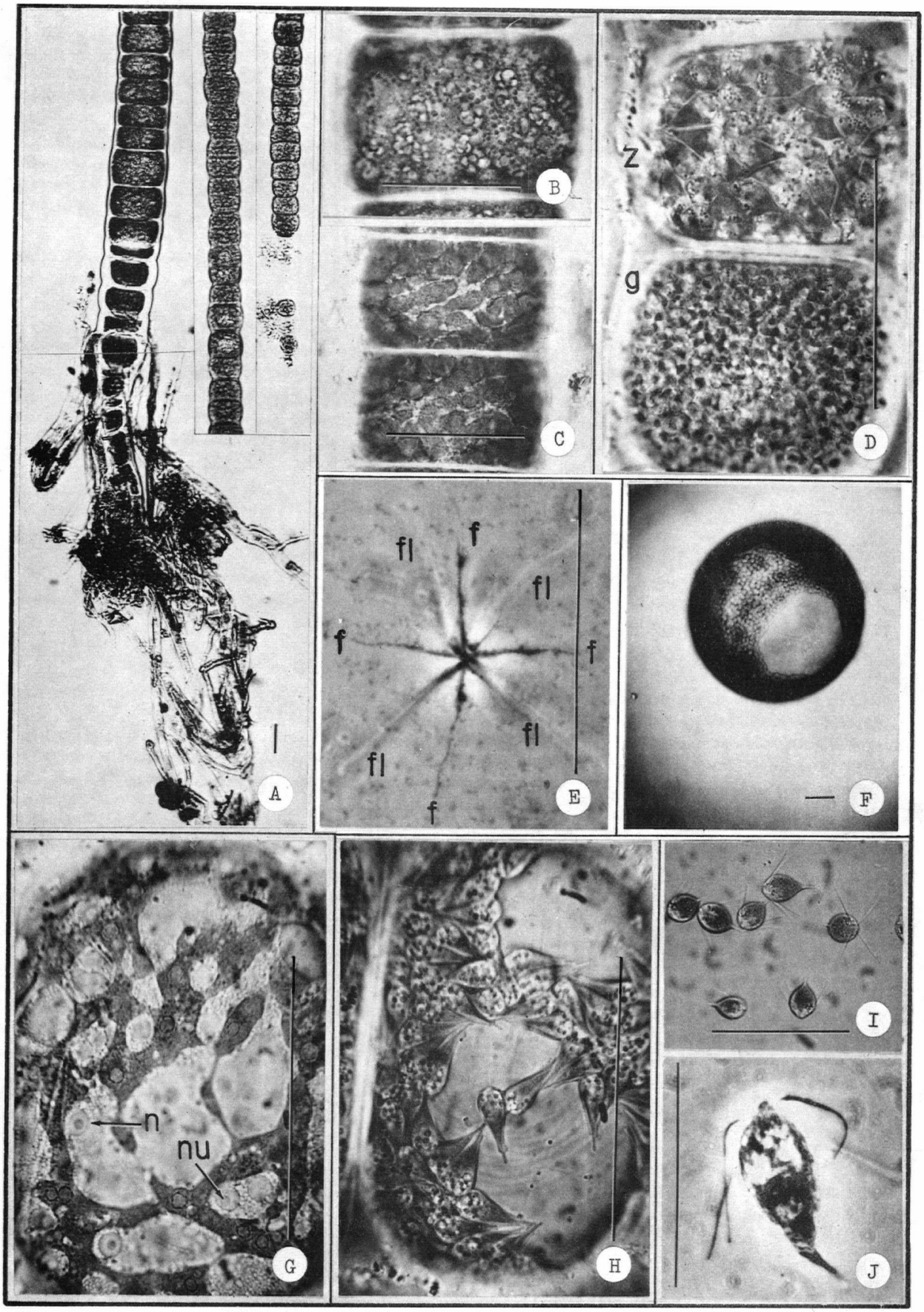


FIGURE 12

Urospora wormskioldii

(Culture, camera lucida drawings)

A - H, N. Sections of one filament.

A. Basal section showing extramatrical rhizoids, x 200.

B. Basal cell showing an early stage in rhizoid formation. x 400.

C. Basal cell showing coarsely-reticulate chloroplast. x 400.

E. Vesicle of zoospores liberated through pore (p) in cell (D). x 400.

F. Germling developing within empty sporangium. x 400.

G,H,N. Sections of filaments showing progressive decrease in filament width towards filament apex. x 400.

I, J. Divaricate rhizoidal ends of another filament. x 400.

K. Female gamete showing asymmetry of body. Composite drawing of living and stained gametes. x 2000.

L. Newly formed spindle-shaped male gamete. x 2000.

M. Gametes in fusion. Aceto-orcein-iron-propiocarmine. x 2000.

Scale length A - J; 50 /u, K - M, 20 /u

FIGURE 12

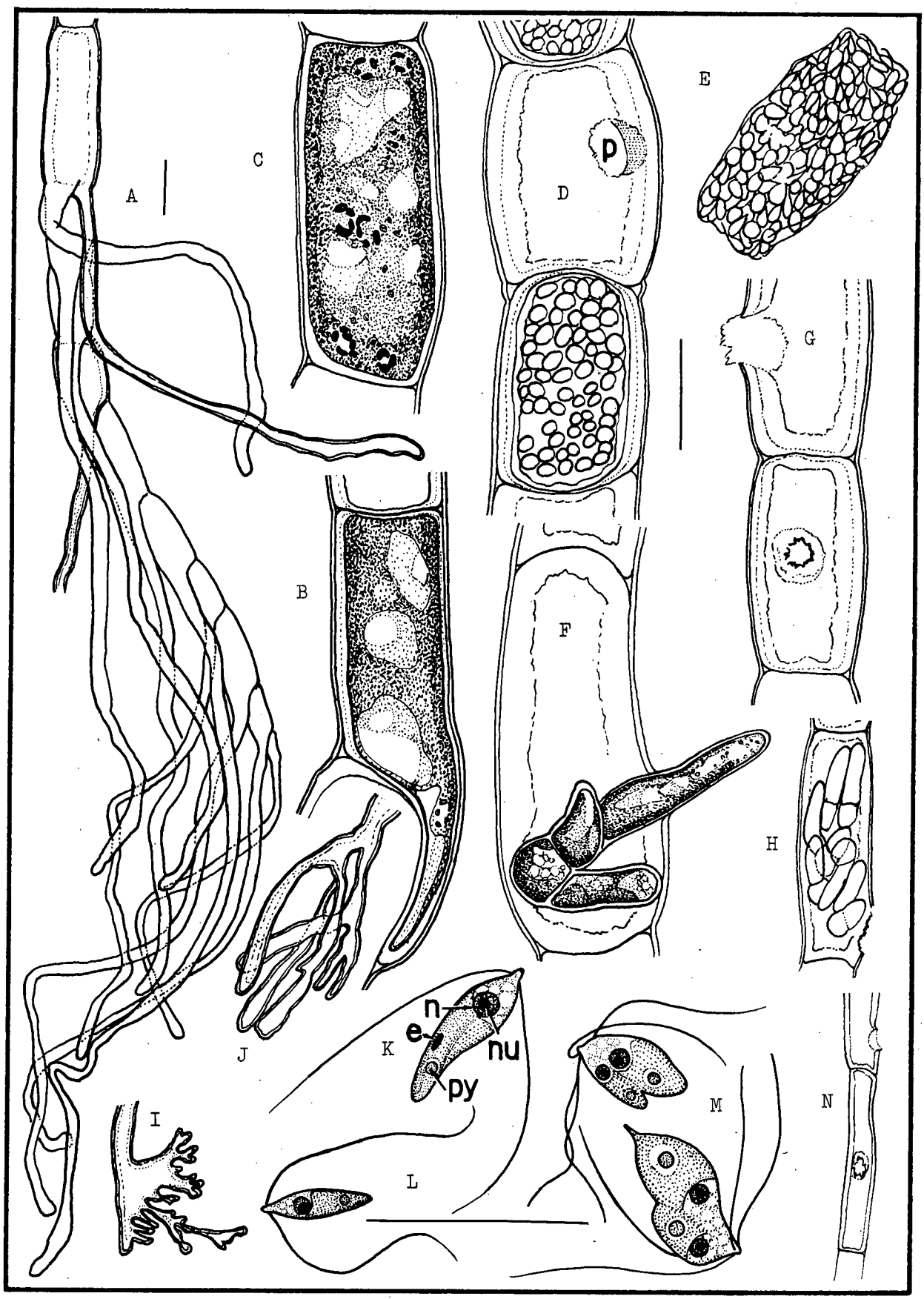


FIGURE 13

Urospora wormskioldii

(Culture)

A. Female filament with gametangia. x 1000.

B, C. Female gametes 12 hours after liberation. Note body asymmetry. B, x 2000. C, x 1000.

D. Female gamete. Aceto-orcein-iron-propiocarmine. x 2000.

E. Female gamete. Flagellar tips much finer. Crystal violet. x 1000.

F. Male filament; gametangium in upper cell; vesicle of male gametes liberated from middle cell. Methylene blue. x 500.

G. Male gametangium with spindle shape of male gametes evident. Methylene blue. x 1000.

H. Abnormal male gamete with two normal flagella and two short flagella. Iron-propiocarmine. x 1000.

I. Abnormal male gamete with four flagella. Flagellar tips much finer. Crystal violet. x 1000.

J. Male gamete with small anterior projection. These are of common occurrence. Aceto-orcein-iron-propiocarmine. x 2000.

K. Newly liberated male gametes with characteristic spindle shape. Phase. x 1000.

Scale length, 25 μ

FIGURE 13

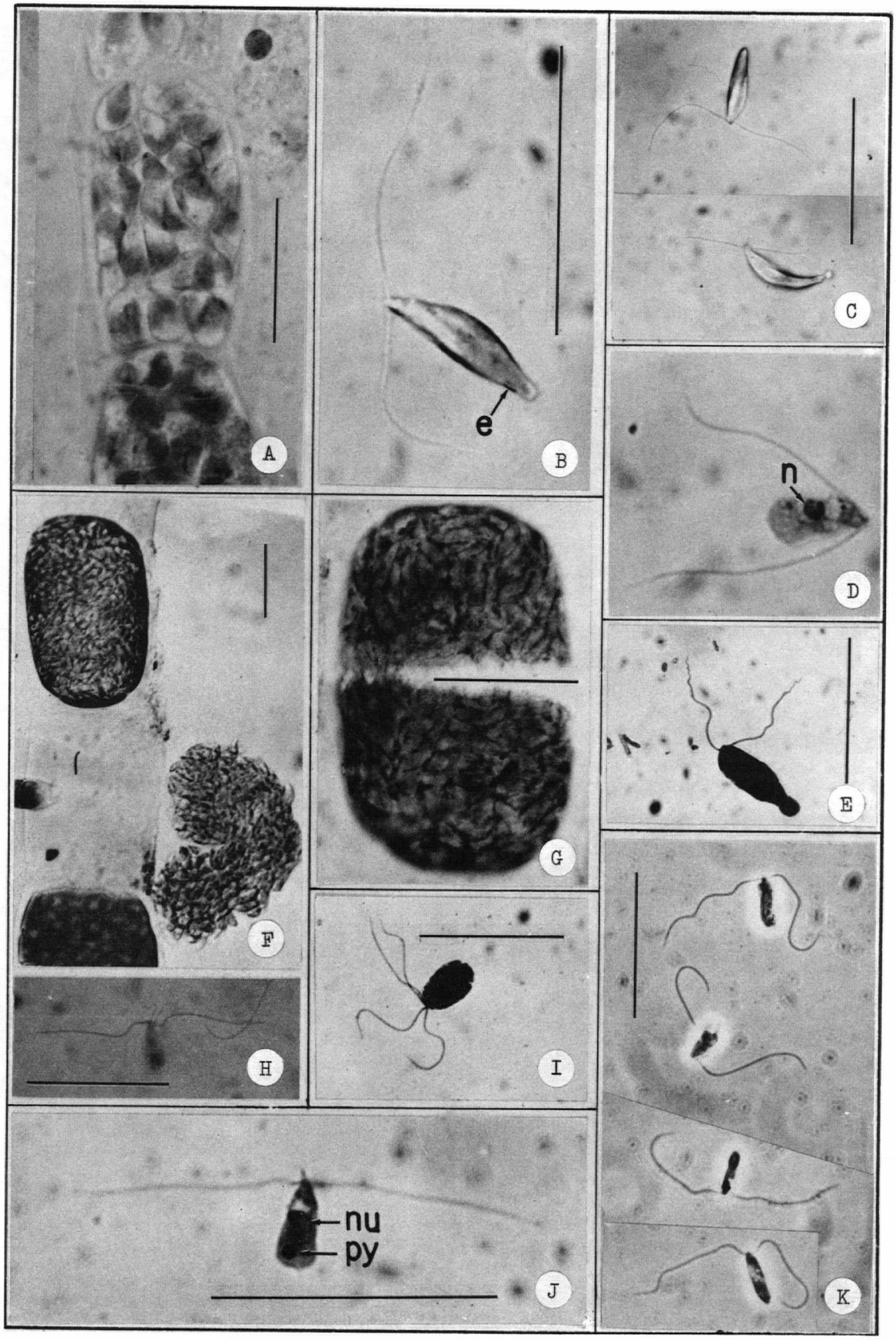


FIGURE 14

Urospora wormskioldii

(A, field. B, C, culture)

A. Stages in gamete fusion. Middle right, two female gametes fusing with one male. Acetocarmine. Phase. x 2000.

B. Codiolum cells from female clone F11; thirty days old. x 100.

C. Codiolum cells from male clone M2A; six months old. Phase. x 200.

Scale length, 25 /u

FIGURE 14

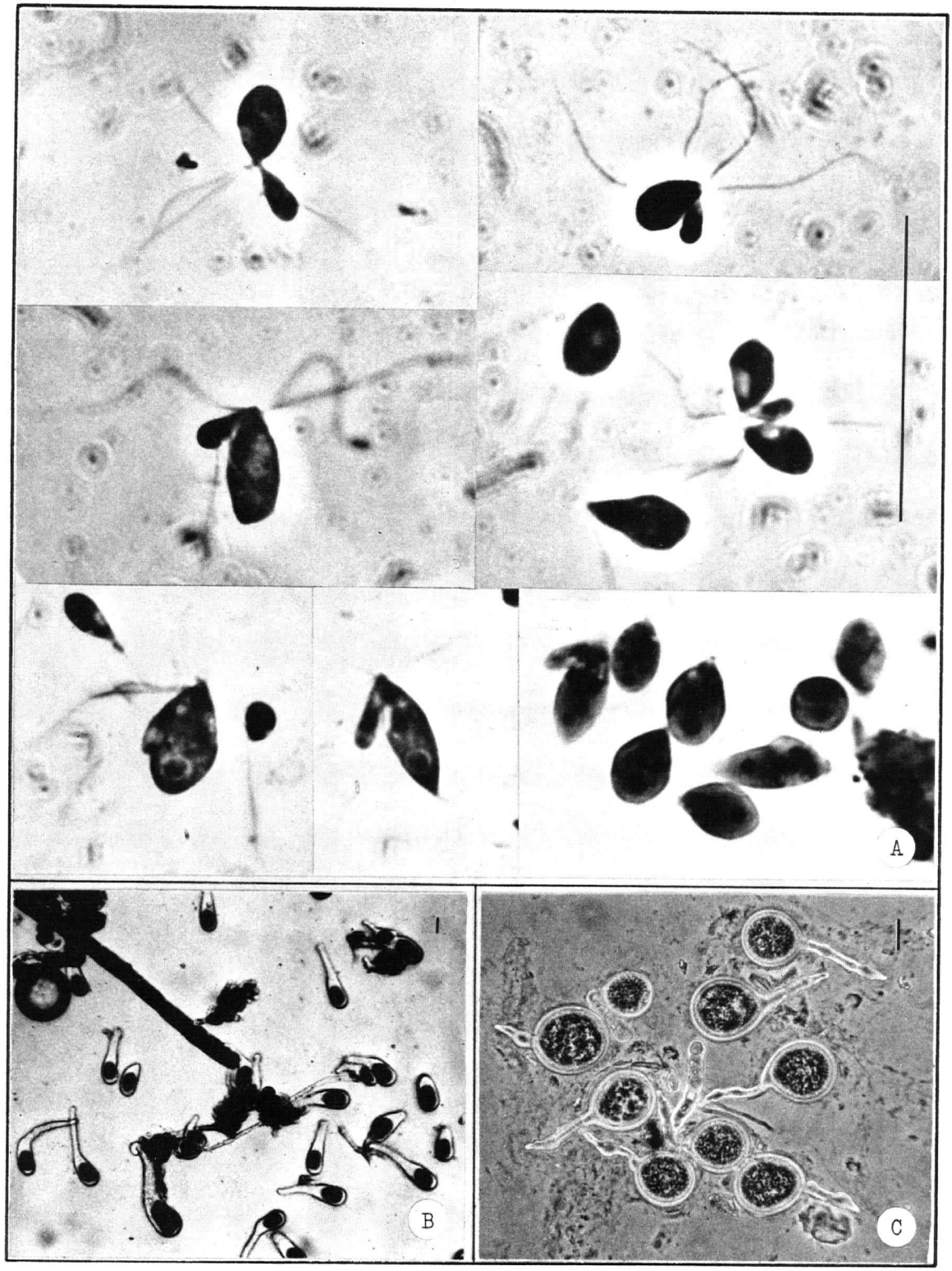


FIGURE 15

LIFE CYCLE OF
UROSPORA VANCOUVERIANA (TILDEN) S. & G.
(TYPE II)

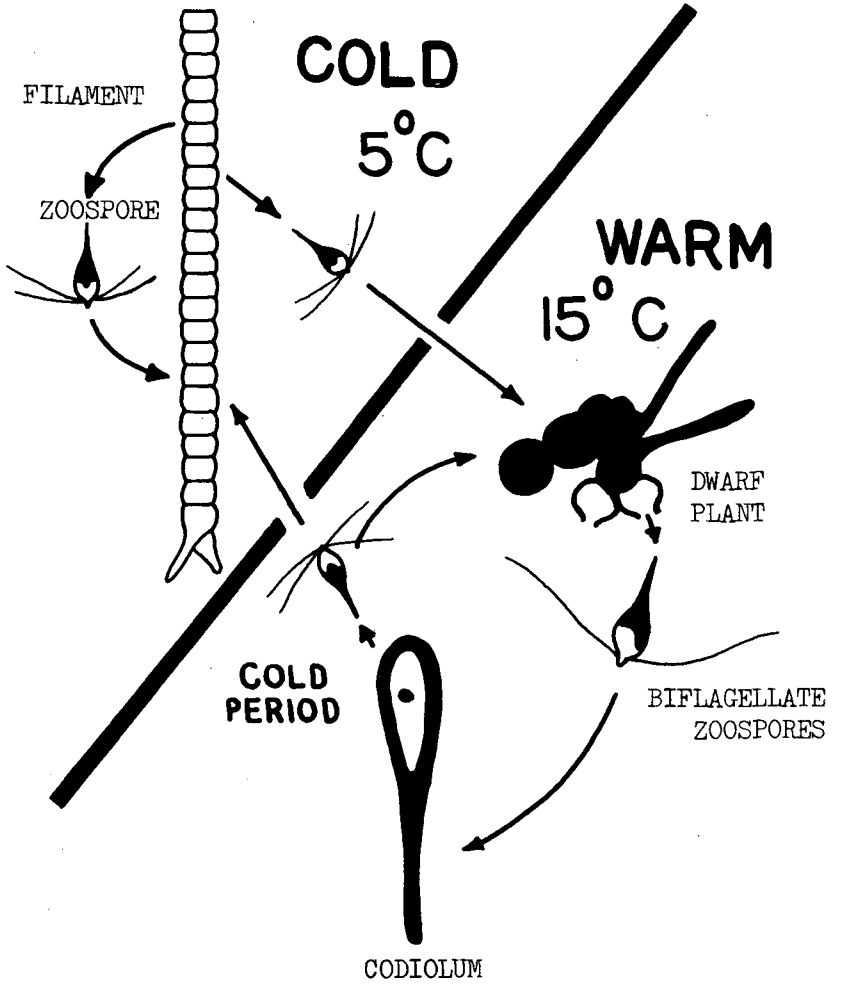


FIGURE 16

EFFECT OF TEMPERATURE ON FILAMENT AND DWARF
PLANT PRODUCTION IN UROSPORA VANCOUVERIANA

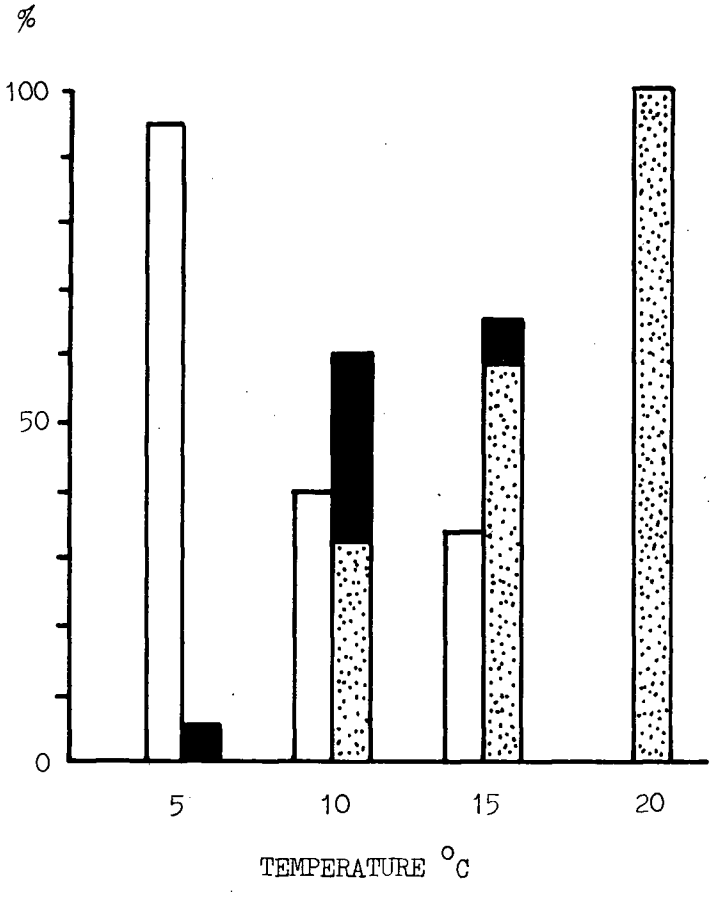


FIGURE 17

Urospora vancouveriana

(A - E, field. F - J, culture)

A. Dry mount of lower half of one filament. Cells of upper half were equal in size to those at the upper end of this filament.

B. Lower part of filament showing holdfast with several intramatrical rhizoids. x 100.

C. Chloroplast of mature cell showing fine perforations. x 500.

D. Surface of fertile sporangium containing quadriflagellate zoospores. x 1000.

E. Surface of fertile sporangium containing quadriflagellate zoospores. x 2000. Insert, x 1000.

F. Filament showing development of lateral rhizoids. x 100.

G. Vegetative cells showing coarsely-perforate chloroplasts. x 500.

H. Empty sporangia showing discharge apertures and ruptured outer sheath. x 300.

I. Holdfast of filament showing several extramatricular rhizoids. x 100.

J. Dwarf plants with fertile sporangia, containing biflagellate zoospores. x 100.

Scale length, 25 μ

FIGURE 17

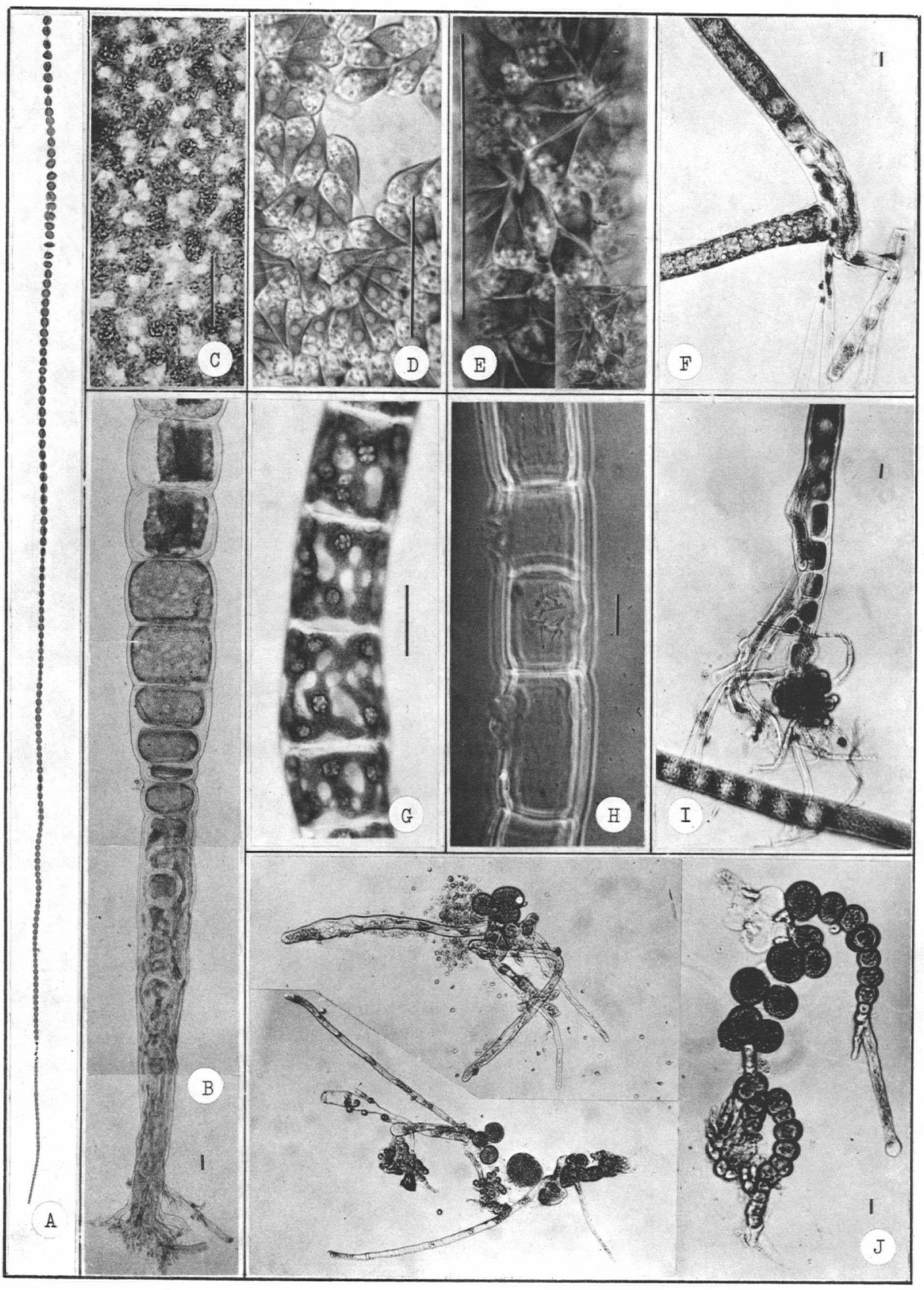


FIGURE 18

Urospora vancouveriana

(Culture)

A. Dwarf plant showing several empty sporangia and one liberated vesicle. x 500.

B. Dwarf plant of an older culture with two sporangia containing quadriflagellate zoospores. x 1000.

C. Dwarf plant with liberated vesicle and several biflagellate zoospores. Note their round appearance. Phase. x 500.

D. Liberation of vesicle containing biflagellate zoospores, two stages. Phase. x 500.

E. Freshly liberated vesicle with biflagellate zoospores. x 1000.

F. Biflagellate spores after 12 hours in the dark. Phase. x 1000.

G. Biflagellate spores stained with iron-propiocarmine. Note the pyrenoid at the basal end, and the fine flagellar tips. x 2000.

H, I. Codiolum cells produced by biflagellate zoospores; twenty-four days old. H, x 100. I, x 500.

Scale length, 25 /u

FIGURE 18

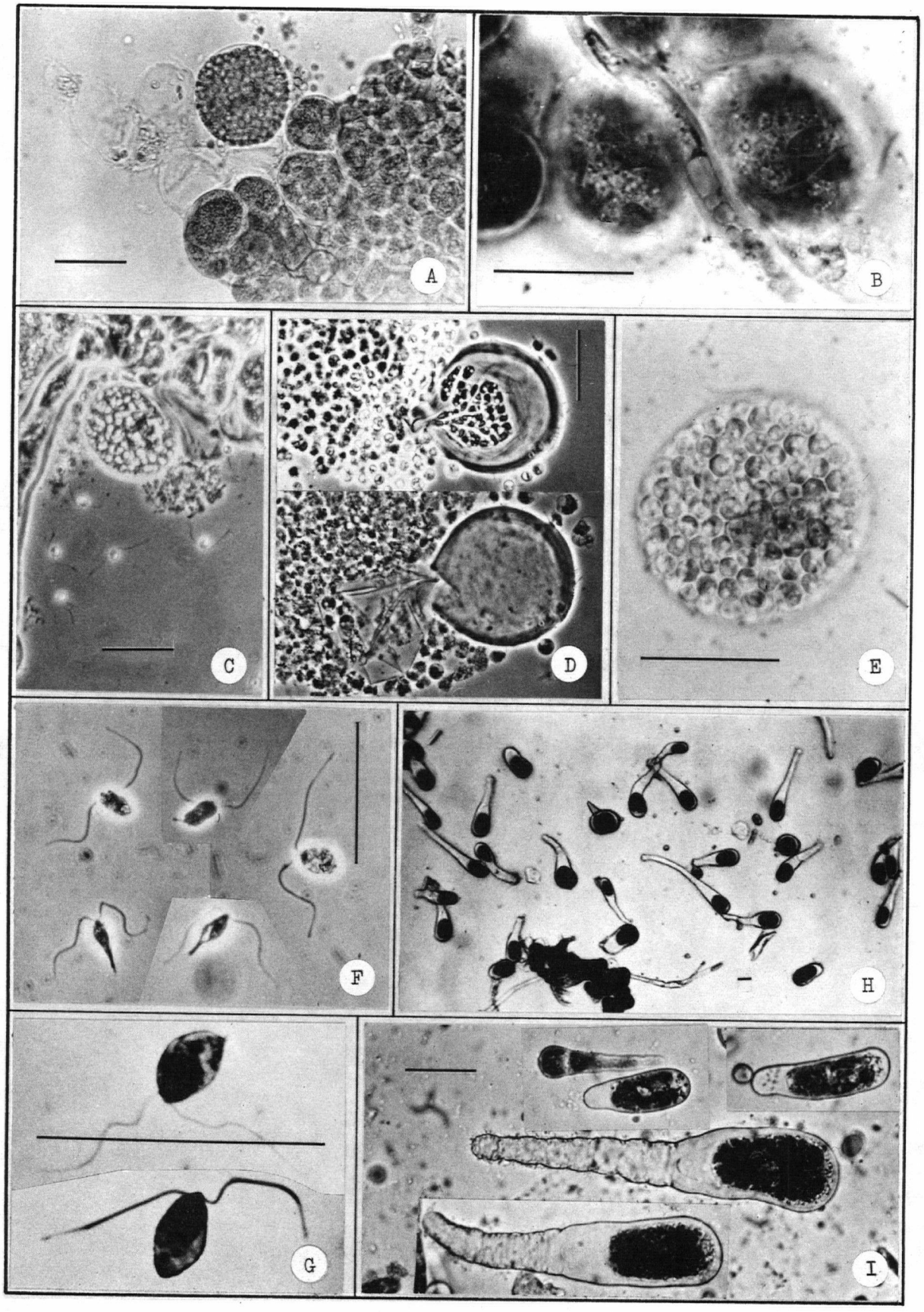


FIGURE 19

Urospora vancouveriana

(Camera lucida drawings of cultural material)

A. Biflagellate zoospores. Iron-propiocarmine. x 2000.

B, C. Germination stages of biflagellate zoospores. Feulgen. x 2000.

D. Codiolum cell; ten days old. Feulgen. x 2000.

E. Codiolum cell; thirty days old. Newcomer-iron-propiocarmine. x 2000.

F. Vesicle of biflagellate zoospores. x 2000.

G. Portion of filament showing concentration of nuclei at end walls and cell middle. Feulgen. x 2000.

H. Young dwarf plant derived from quadriflagellate zoospores. Feulgen. x 2000.

I. Dwarf plant with one cell undergoing division. Feulgen. x 1000.

FIGURE 19

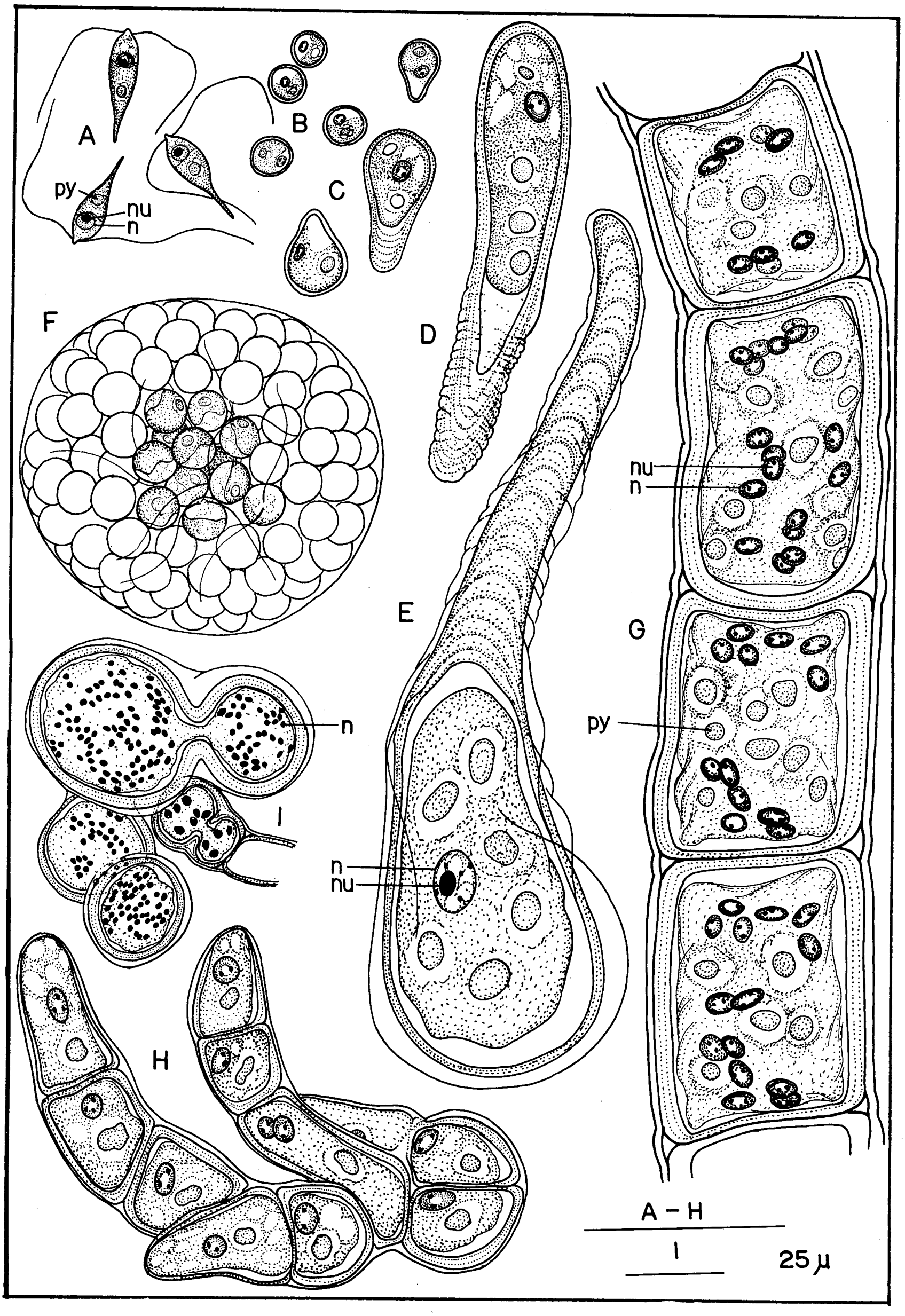


FIGURE 20

Urospora vancouveriana

(Culture material stained with Feulgen-iron-propiocarmine)

A. Germlings from quadriflagellate zoospores. Nucleolus unstained. Note feulgen positive chromatic bodies (cb). x 2000.

B. Pre-sporangial cells of dwarf plant; upper cell with nuclei in metaphase. x 1000.

C. Cells of dwarf plant; upper left cell with nuclei in prophase; remaining cells with nuclei in interphase. x 1000.

D. Apical portion of filament; upper cell with nuclei in metaphase; lower cell with nuclei in interphase. x 1000.

E. Apical cell of filament much squashed; nuclei in metaphase. Inserts prepared with the aid of camera lucida drawings. x 1000.

F. Metaphases in a filament cell. Figure on left prepared with the aid of camera lucida drawings. x 3000.

G. Filament showing multinucleate condition of each cell. Nuclei (n) are lightly stained and contain several Feulgen-positive chromatic bodies (cb). x 500.

H. Metaphase in a filament cell showing some chromosomes to be elongate. x 3000.

I. Rhizoidal nuclei (n), showing conspicuous nucleoli (nu) and 2-3 Feulgen positive chromatic bodies. x 2000.

Scale length A - F, 25 /u; G, 50 /u; H, I, 10 /u

FIGURE 20

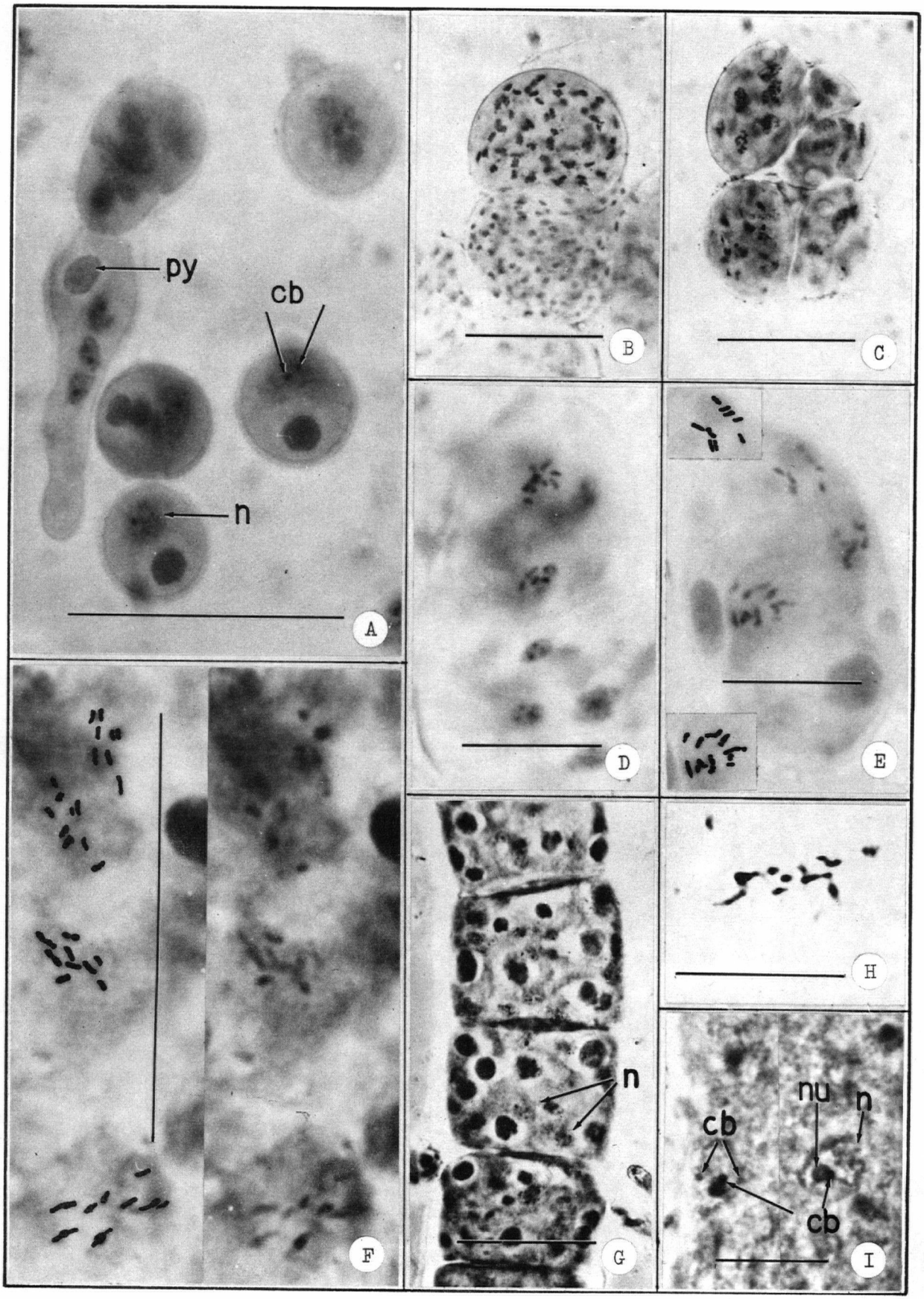


FIGURE 21

A. Urospora wormskioldii. A cell in early stage of zoospore formation showing clusters of nuclei. Feulgen-iron-propiocarmine. x 1000.

B. U. wormskioldii. A fertile cell with immature zoospores. Feulgen-iron-propiocarmine. x 1000.

C. U. vancouveriana. A fertile cell with mature zoospores. Note the anterior projections of the chloroplast (ch). Feulgen-iron-propiocarmine. x 1000.

D. U. wormskioldii. Plasmolized zoospores showing swellings (s) at the base of flagella. x 2000.

E. U. wormskioldii. A germling with pyrenoids (py) appearing dissected. Feulgen-iron-propiocarmine. 10/u section. x 2000.

F. Codiolum gregarium. A cell with pyrenoids (py) appearing dissected. Feulgen-iron-propiocarmine. 10/u section. x 1000.

G. U. speciosa. Fertile field filament showing liberation of zoospores in a vesicle. x 500.

H. U. speciosa. Cultural filaments showing their uninucleate condition. Newcomer-iron-propiocarmine. x 1000.

Scale length, 25/u

FIGURE 21

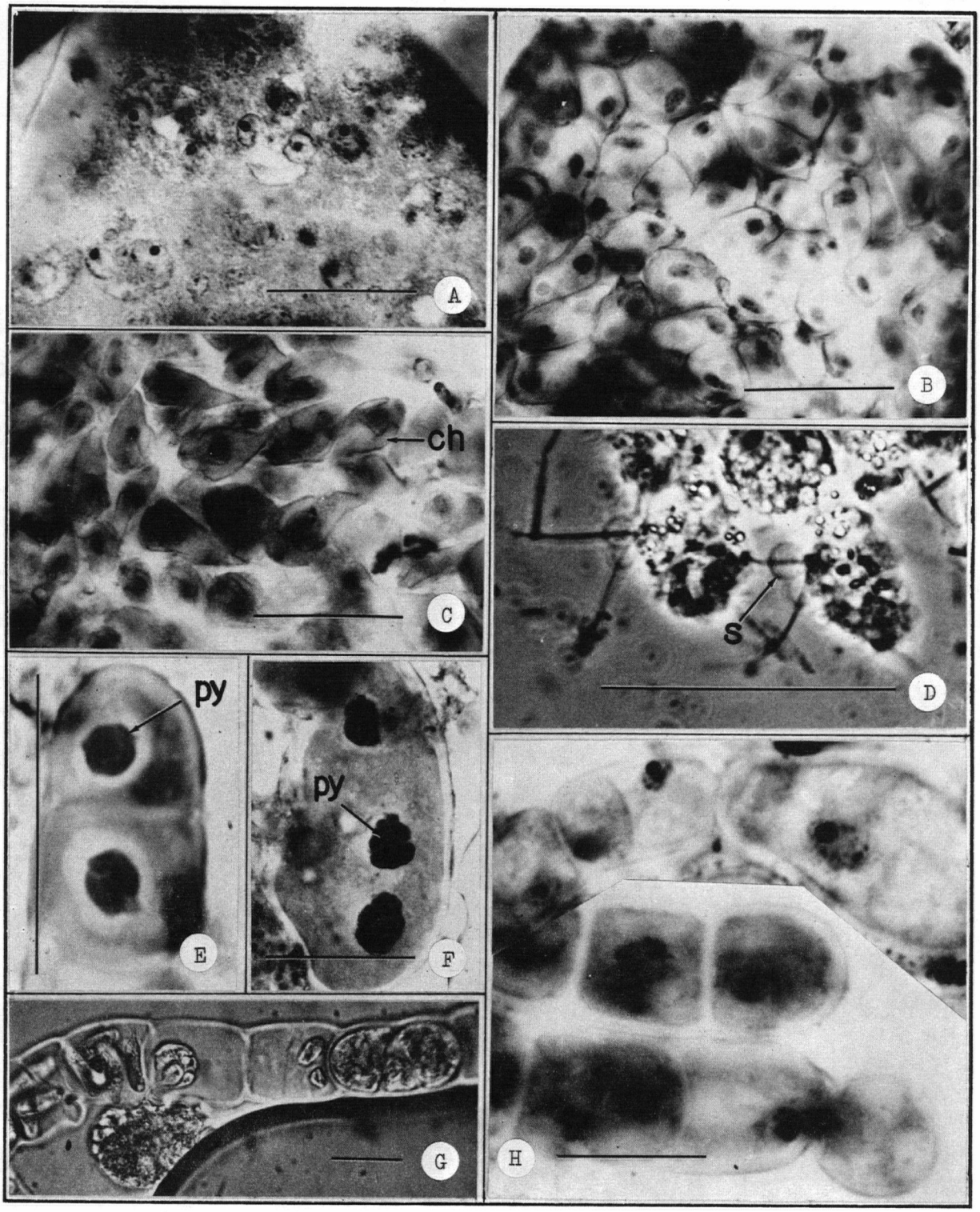


FIGURE 22

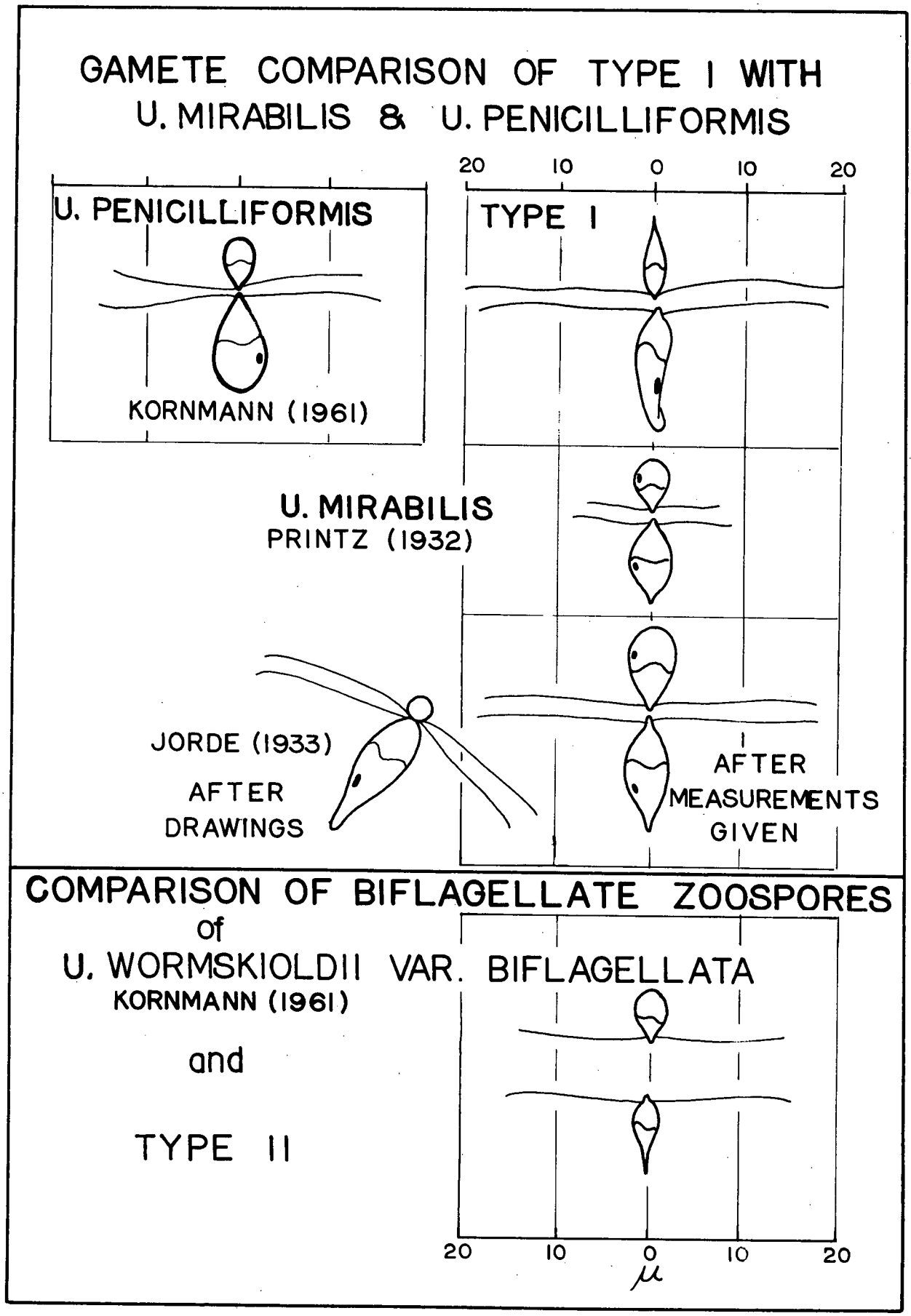

GAMETE COMPARISON OF TYPE I WITH
U. MIRABILIS & U. PENICILLIFORMIS
U. PENICILLIFORMIS
KORNMANN (1961)
20 10 0 10 20
TYPE I
U. MIRABILIS
PRINTZ (1932)
JORDE (1933)
AFTER
DRAWINGS
AFTER
MEASUREMENTS
GIVEN
COMPARISON OF BIFLAGELLATE ZOOSPORES
of
U. WORMSKIOLDII VAR. BIFLAGELLATA
KORNMANN (1961)
and
TYPE II
20 10 0 10 20
μ

FIGURE 23 VARIATION AND MATINGS IN UROSPORA WORMSKIOLDII

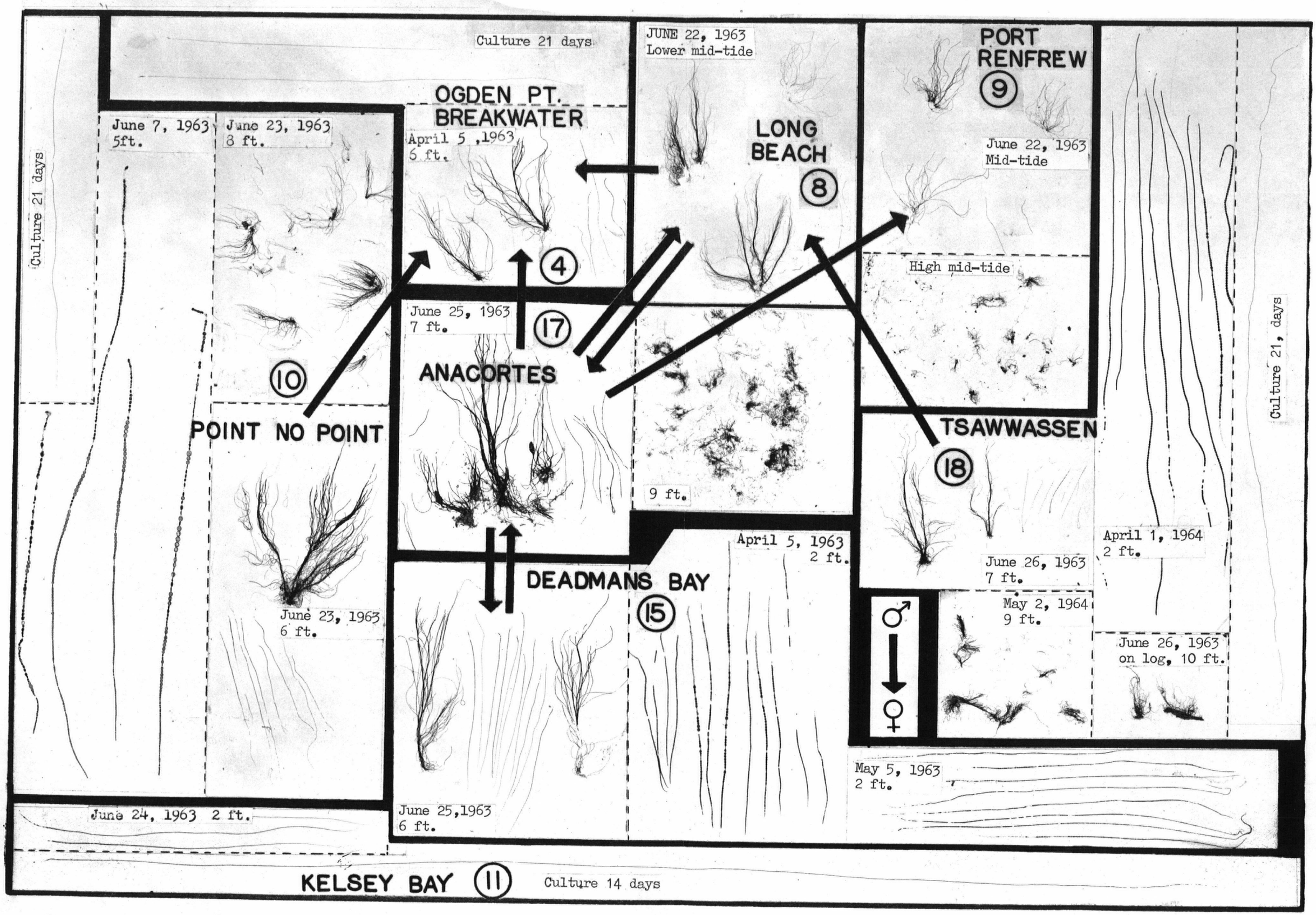


FIGURE 24

A. Codiolum gregarium from Deadman's Bay, February 1961. Nearly fertile cell showing pectic lamellations of the stipe. Composite photograph. Acetocarmine. x 300.

B. C. gregarium. A fertile cell with quadriflagellate zoospores. x 800.

C. C. gregarium zoospores germinating within the cell. IKI and eosine. x 400.

D. C. pusillum from Oak Bay, October 9, 1963. Clava elongate with cell gradually tapering towards the base. Living. x 100.

E. C. gregarium from Friday Harbor, August 30, 1963. Clava swollen with transition between clava and stipe abrupt. Living. x 100.

Scale length, A - C, 50 /u; D, E, 500 /u

FIGURE 24

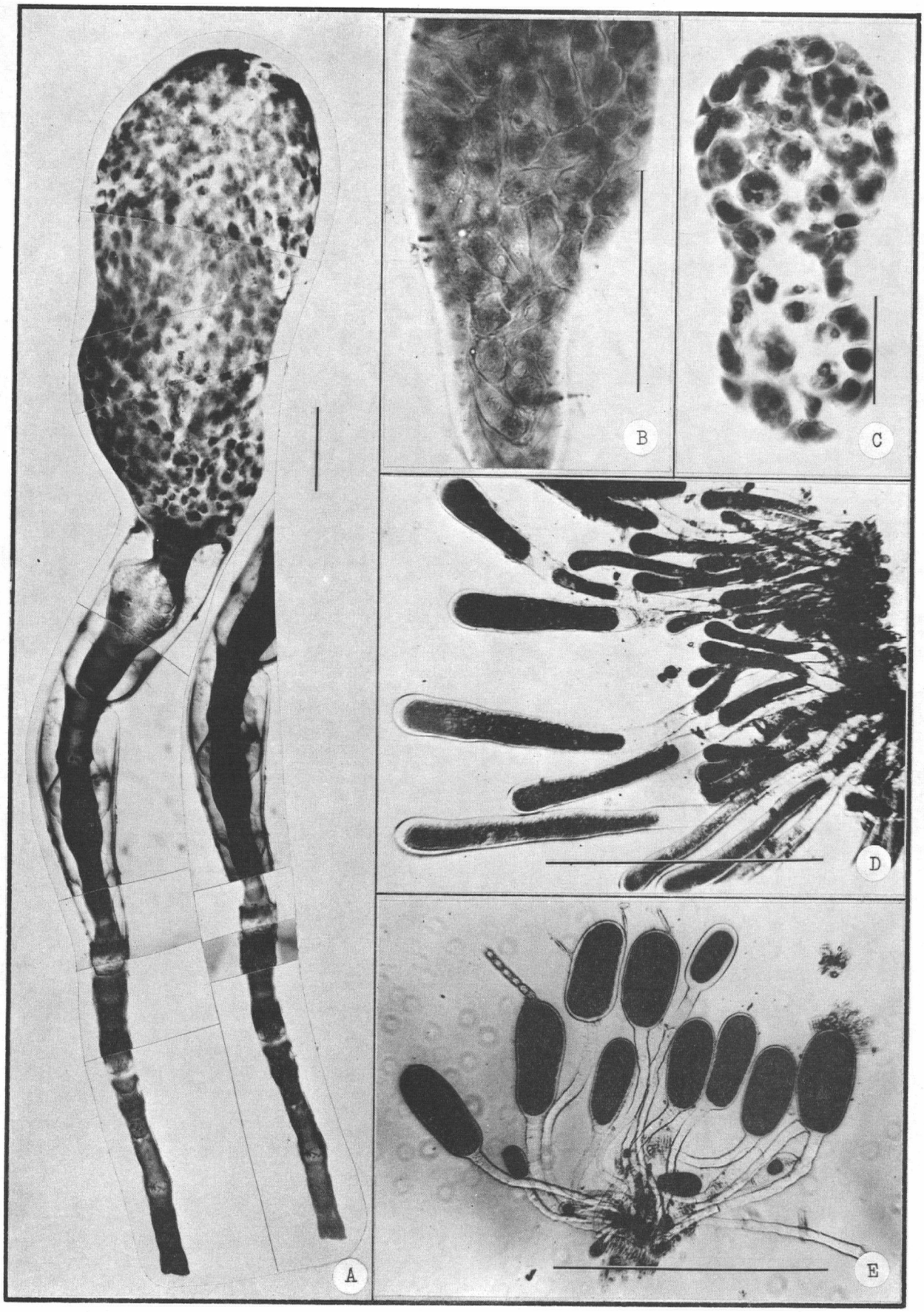


FIGURE 25

Codiolum gregarium and cultural products

A. Codiolum gregarium from Deadman's Bay, February 1961. A vegetative cell showing uninucleate condition. Only the nucleolus is evident. Acetocarmine. x 1000.

B. Quadriflagellate zoospores from C. gregarium. Note anterior projections of the chloroplast. Acetocarmine. x 2000.

C - E. Developmental stages of germinating C. gregarium zoospores.

C. Attached zoospore, flagella lost. Acetocarmine. x 2000.

D. Two-cell stage with zoospore tail still present. Acetocarmine. x 2000.

E. Three-cell stage with lower cell developing into a rhizoid. Acetocarmine. x 2000.

F. Germling showing filament and dwarf characteristics; upper five cells are filament-like; lower five cells are dwarf-plant-like. Acetocarmine. x 1000.

G. Young dwarf plant showing multinucleate condition and irregular arrangement of cells. Acetocarmine. x 1000.

Scale length, 25 μ

FIGURE 25

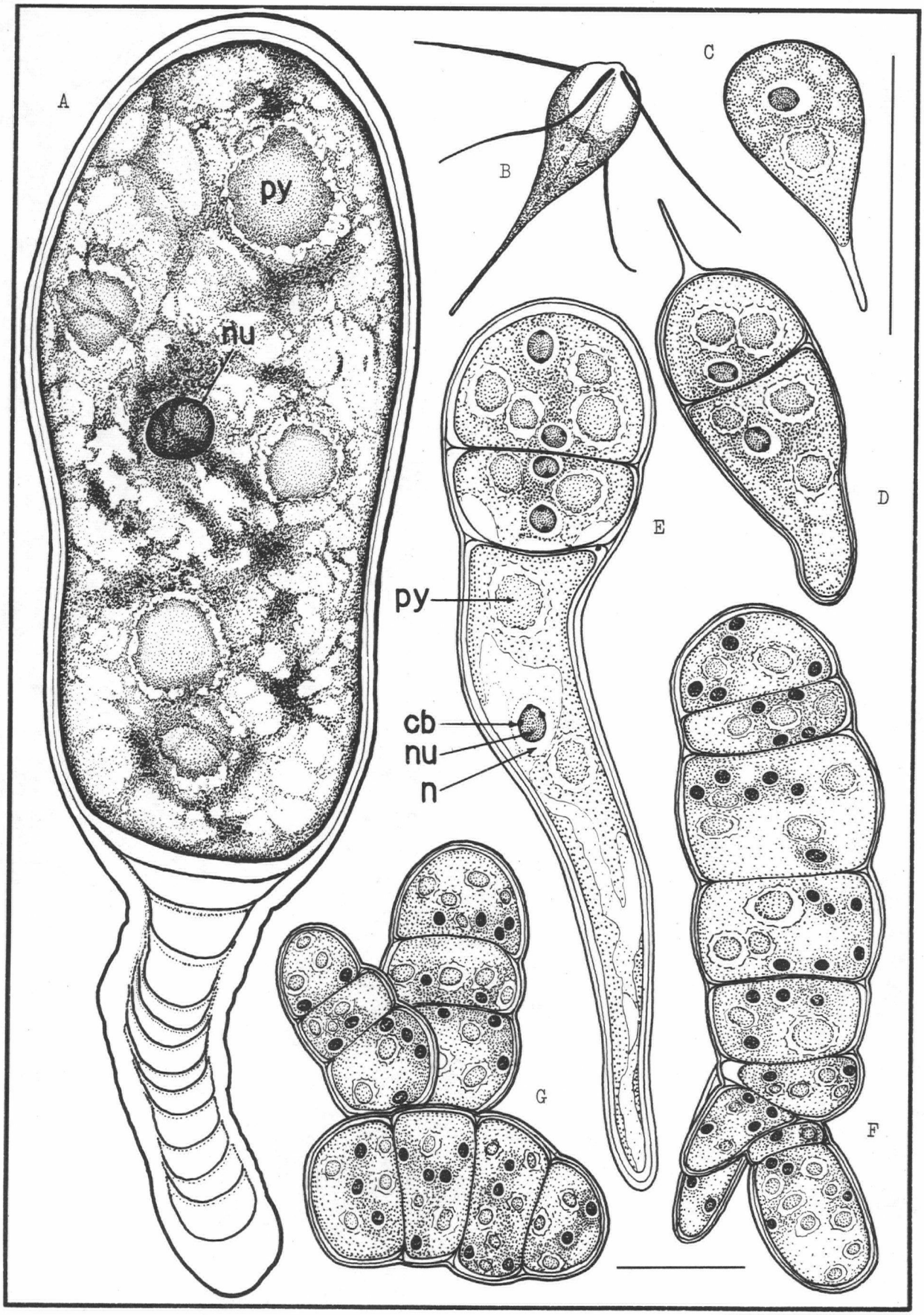


FIGURE 26

A - C. Codiolum gregarium in first division metaphase showing ten (?) chromosome pairs. Acetocarmine. A, x 600. B, camera lucida. x 3000. C, x 3000.

D. C. gregarium interphase nucleus showing coarse reticulation and nucleolus with large central vacuole. Newcomer-iron-propiocarmine. x 1000.

E. C. pusillum in first division metaphase showing thirteen chromosomes or twelve chromosomes and one nucleolus. Newcomer-iron-propiocarmine. x 2000.

F. C. gregarium at the two-nucleate stage, with only the nucleoli evident. Acetocarmine. x 500.

G. C. gregarium in third division metaphase. Insert is a higher magnification of rectangle showing eleven chromosomes. Acetocarmine. G, x 500. Insert, x 3000.

H. C. gregarium in ninth division metaphase. One optical plane showing about 25 of the 226 nuclei seen; others were obscured by foreign material. Acetocarmine. x 500.

I - J. C. pusillum subjected to daily thermoperiod. Optical sections of one nucleus showing five nucleoli-like structures and several smaller chromosome-like bodies (arrows). Living. x 1000.

K. One metaphase plate from cell (H) showing ten chromosomes. Camera lucida. Acetocarmine. x 3000.

L - N. Zoospores of Urospora wormskioldii in first nuclear division showing eleven chromosomes. Colchicine arrested. N, camera lucida drawing of (M). Acetocarmine. L, x 1000. M, N, x 2700.

O. C. pusillum in the four-nucleate stage showing a nucleus with a prominent nucleolus and several chromosome-like bodies (arrows). Living. x 1000.

P. C. pusillum, interphase nucleus with prominent vacuolated nucleolus. Living. x 1000.

Q - R. U. wormskioldii germling from a C. gregarium zoospore, colchicine arrested, ten chromosomes visible. R, is a higher magnification of rectangle in (Q). Acetocarmine. Q, x 1000. R, x 3000.

Scale length, 10 μ

FIGURE 26

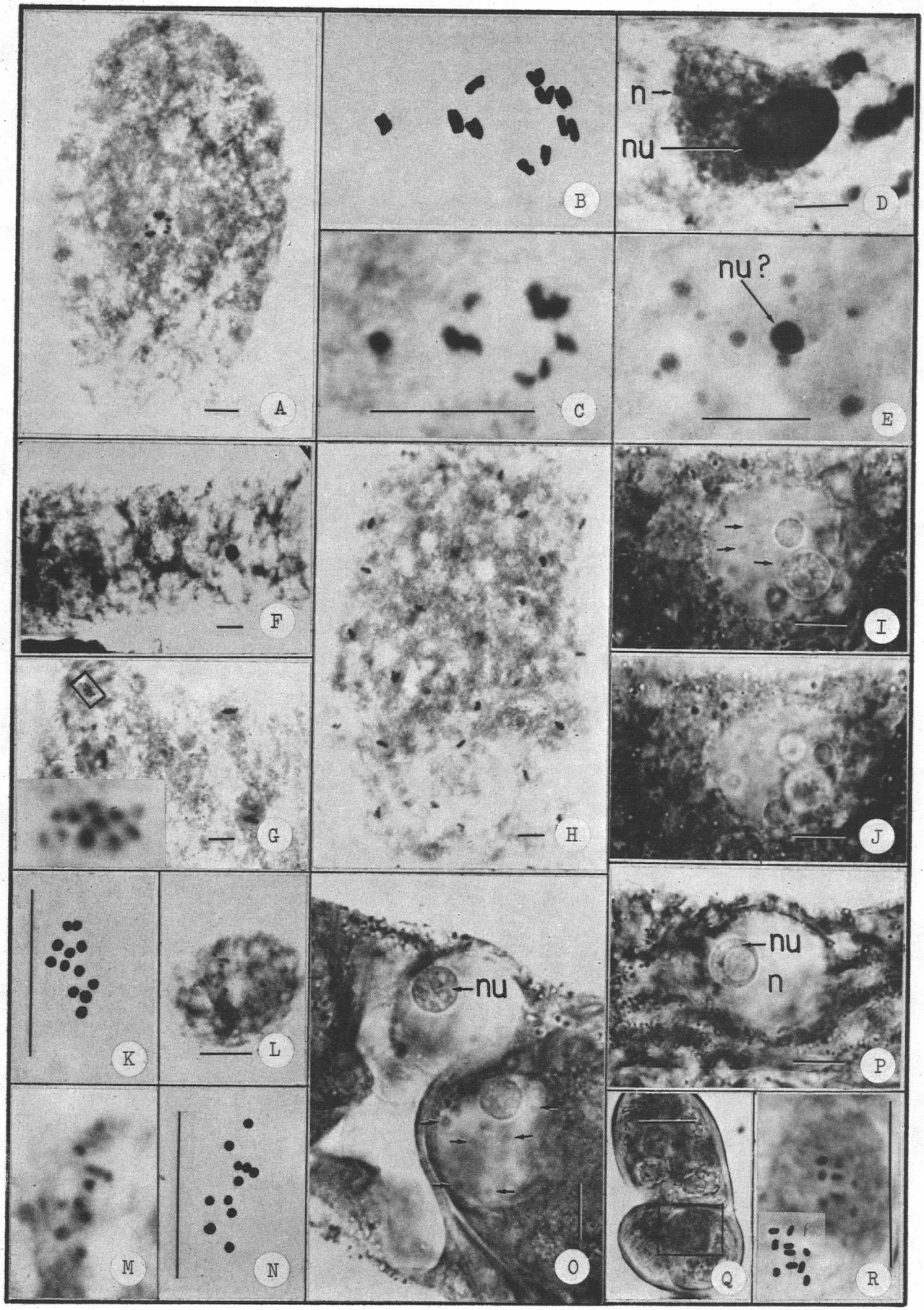


FIGURE 27

Urospora wormskioldii cultural filaments derived from Codiolum gregarium zoospores

(Camera lucida drawings)

A - D. Sections of one filament.
A. Basal section showing intramatrical rhizoids and divaricate rhizoidal ends. x 200.

B-D. Distal sections of the same filament showing decrease in filament width. x 200.

E, F. Cells of filament showing multinucleate condition. Acetocarmine. x 800.

G. Interpretive drawing of wall structure during spore release. An additional inner wall appears to be formed during spore development. x 600 (approx.)

H. Rhizoidal growth, showing middle wall formed last. x 1500.

I. Zoospore showing nuclear area unstained and prominent nucleolus. Acetocarmine. x 3000.

J - L. Zoospores showing flagellar insertion between zoospore ridge fibrils (f) and the four anterior projections of the chloroplast. x 3000.

Scale length, A - G, 50 /u; I - K, 25 /u

FIGURE 27

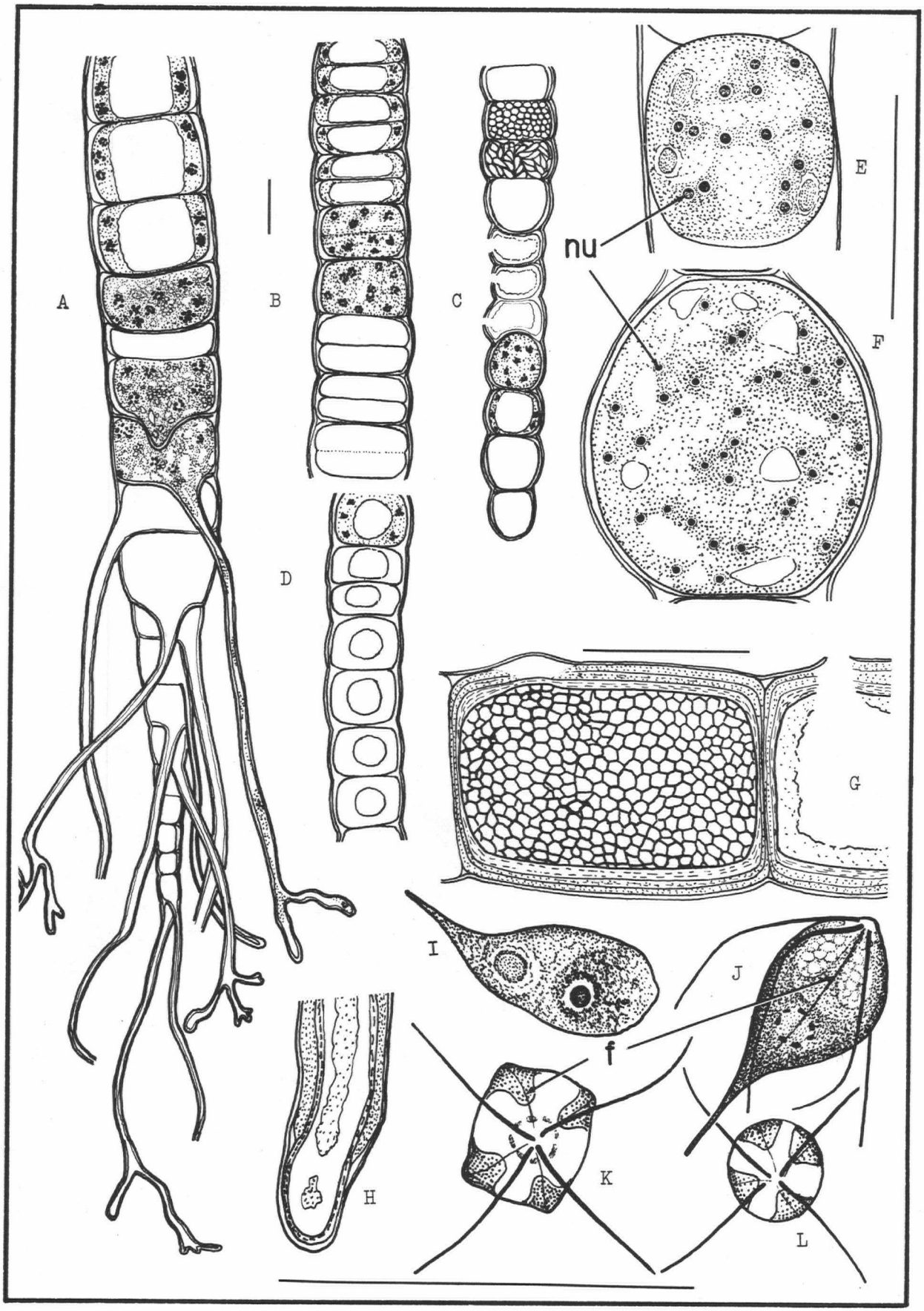


FIGURE 28

Codiolum gregarium fertility in nature and after transplantation

A. Ladder on the old dock at the Friday Harbor Marine Laboratories, lowered at (2) with weight (1) on the bottom. Rungs positioned every three feet with the lowest at the minus nine foot and the highest at the twelve foot tidal level.

B. Log below the new laboratory building from which the Codiolum samples were removed. Codiolum between arrows.

C. Ladder rung with six samples stapled to the front and back surface of rung.

D. Sampled area of log.

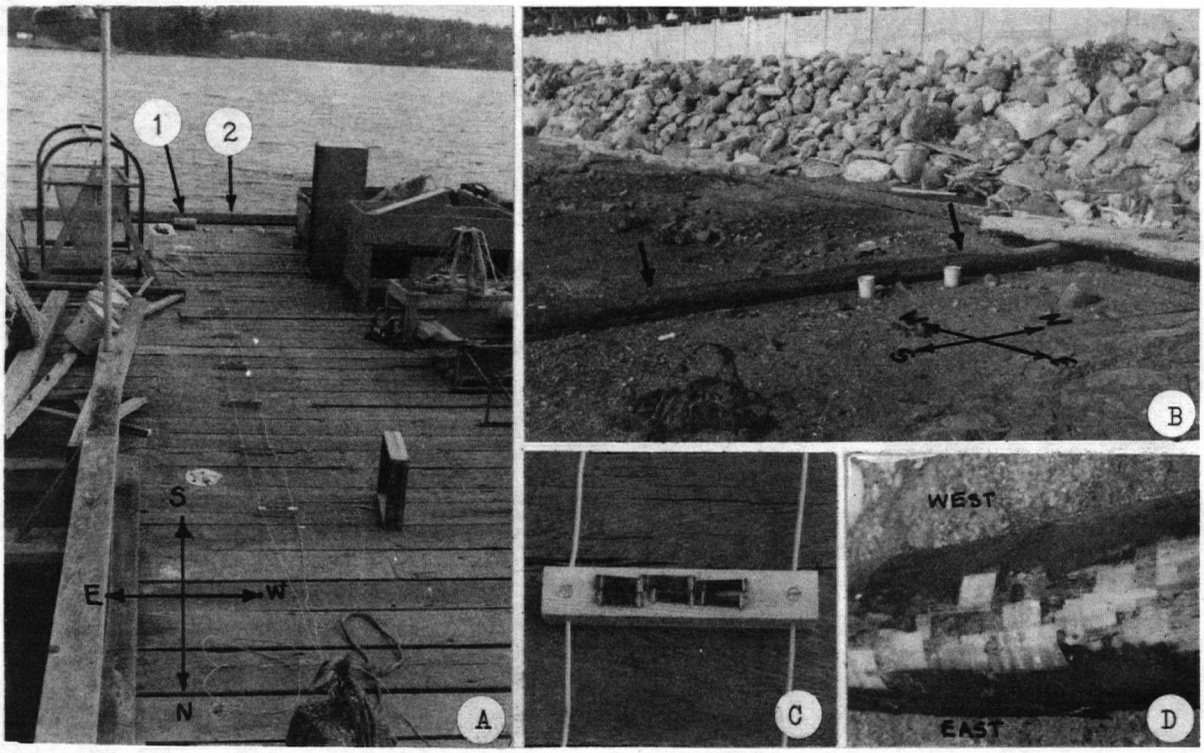
E. Tabular results of transplant effect on Codiolum fertility.

F. Fertility of Codiolum on log in (B).

G. Fertility of Codiolum on rock at Deadman's Bay.

FIGURE 28

TRANSPLANTATION EFFECT ON CODIOLUM FERTILITY



Aug. - Sept. TRANSPLANTATION EFFECT E

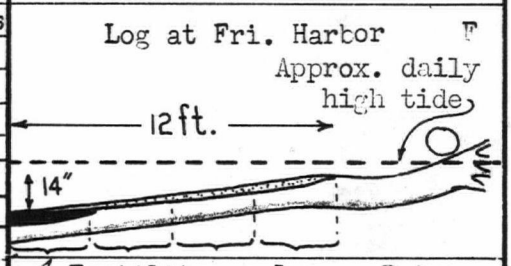
30 29 % Fertility or nuclear divisions

DAY	0	8		16		30	
ft.		F	ND	F	FD	F	FD
12.0 N/S	DAY OF TRANSPLANT - AUG. 30						
9.0 N/S			1	2/2		15/8	
6.0 N/S			1/1	5/10	xxx	20/22	xxxx
3.0 N/S		1/2	1/3	4/4	xxx	100/20	xxx
0.0 N/S			1/1	2/4	x	a/20	x
-3.0 N/S				4/3		a/a	
-6.0 N/S				3/8		a/a	
-9.0 N/S				9/7		100/a	

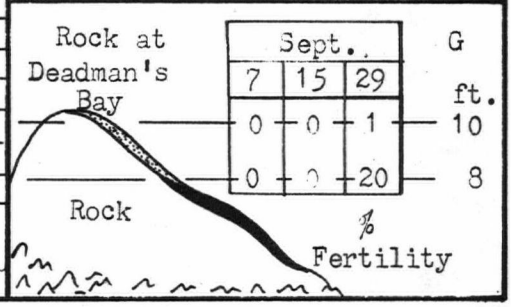
TEMPERATURE °C

ft				
Air	27.7 (1 PM)	18 (12 Noon)	14.5
1 water	11.6 "	11	11.5
10 "	10.6 "	11	10.3

FERTILITY IN NATURAL HABITAT



% Fertility on Log				Date
0	0	0	0	Aug.30
0	0	0	0	Sept.7
-	0.3	-	6	Sept.15
20	1.5	50	30	Sept.29



Urospora, Codiolum, FD - filamentous diatoms, x - sparse, xxx - dense

ND - nuclear divisions, a - Codiolum absent

FIGURE 29

Codiolum cells from Urospora wormskioldii female clone 8L, fertility induced with cold shock. Living material.

A. Cell with two Codiolum cells, rounded up zoospores, one cell with an eyespot (e) and one large donut-shaped mass. x 2250.

B. Cell with rounded-up structures and one zoospore with two flagella. x 2250.

C - E. Cells forming irregular masses. C, x 2250. D, E, x 1000.

FIGURE 29

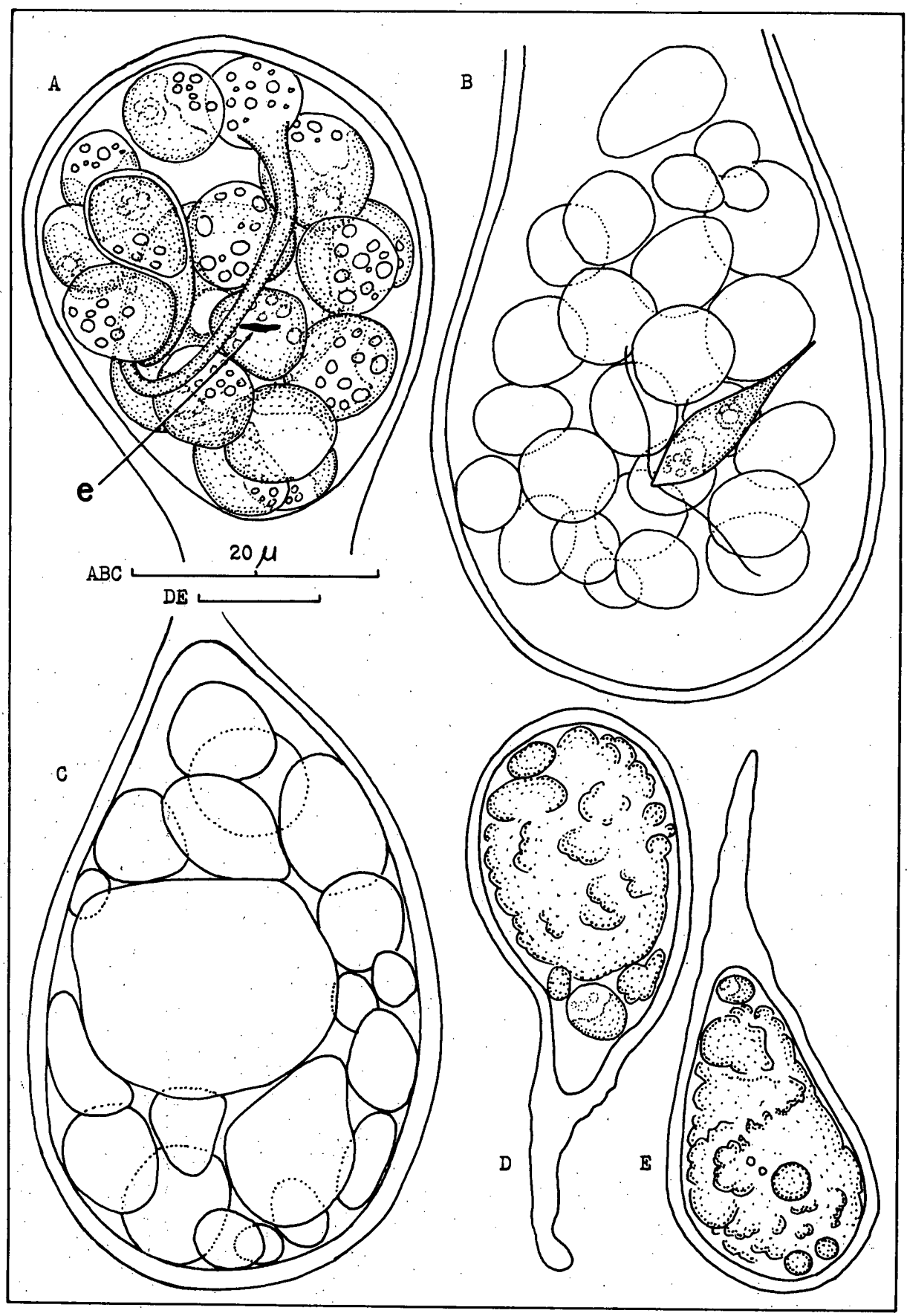


FIGURE 30

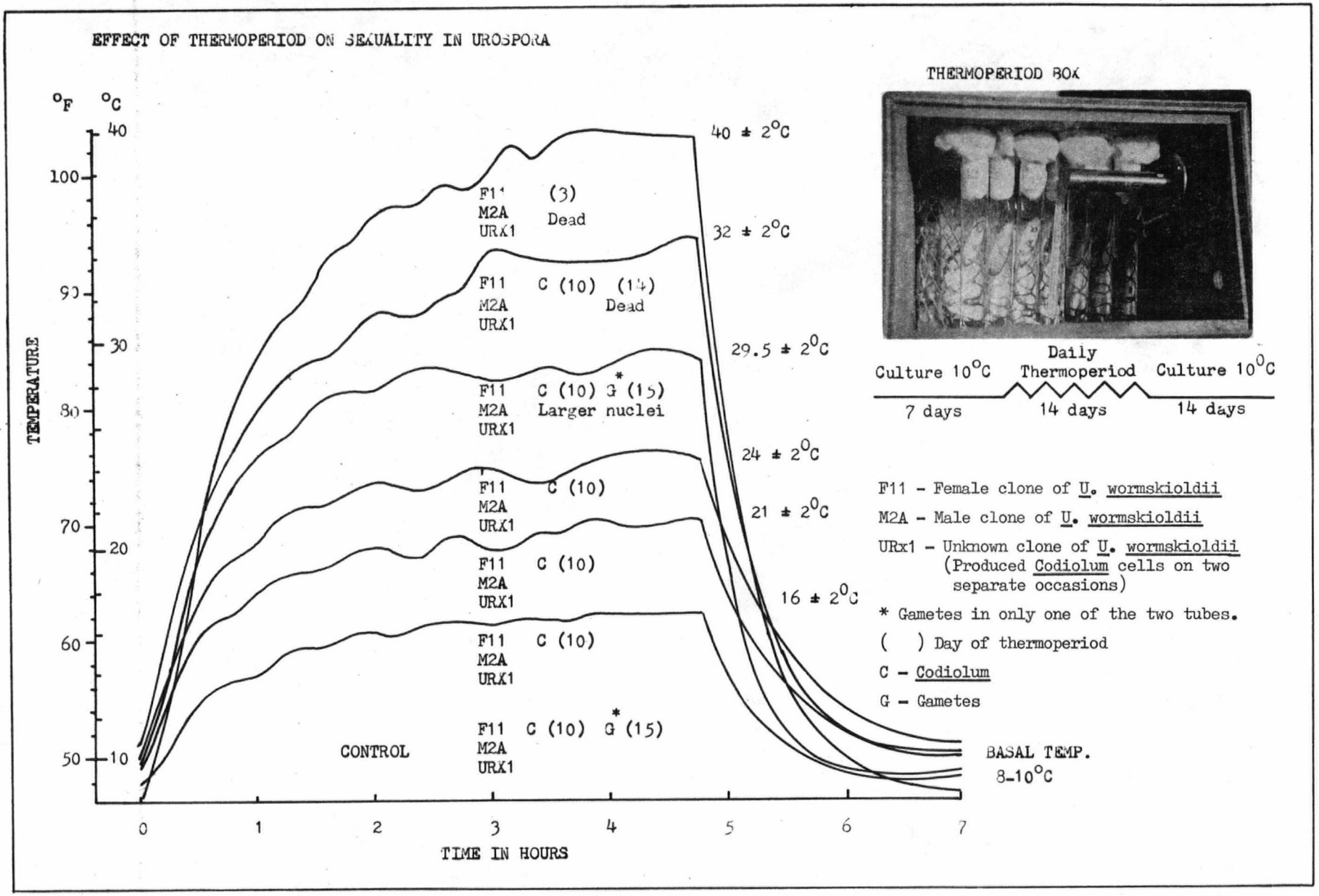
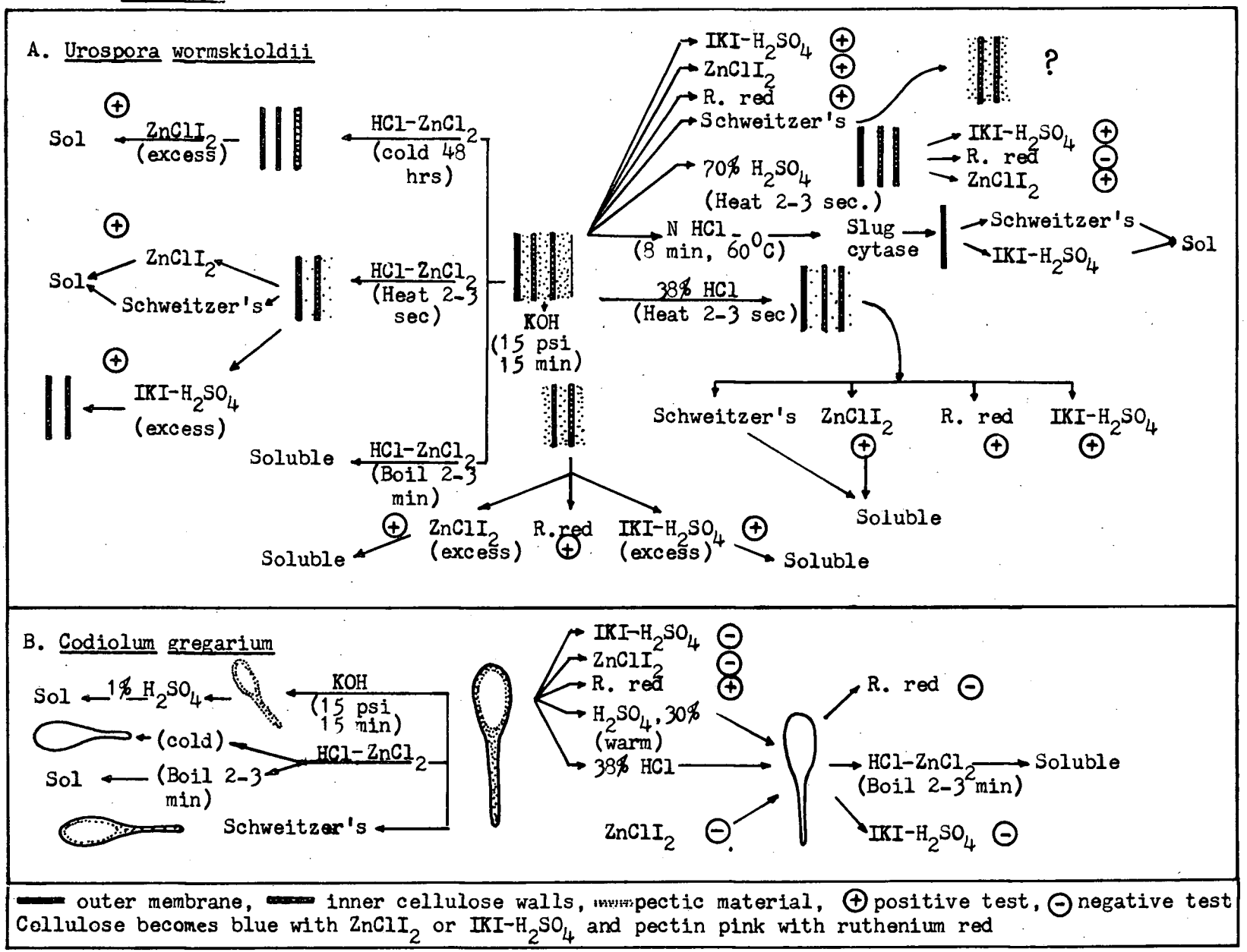
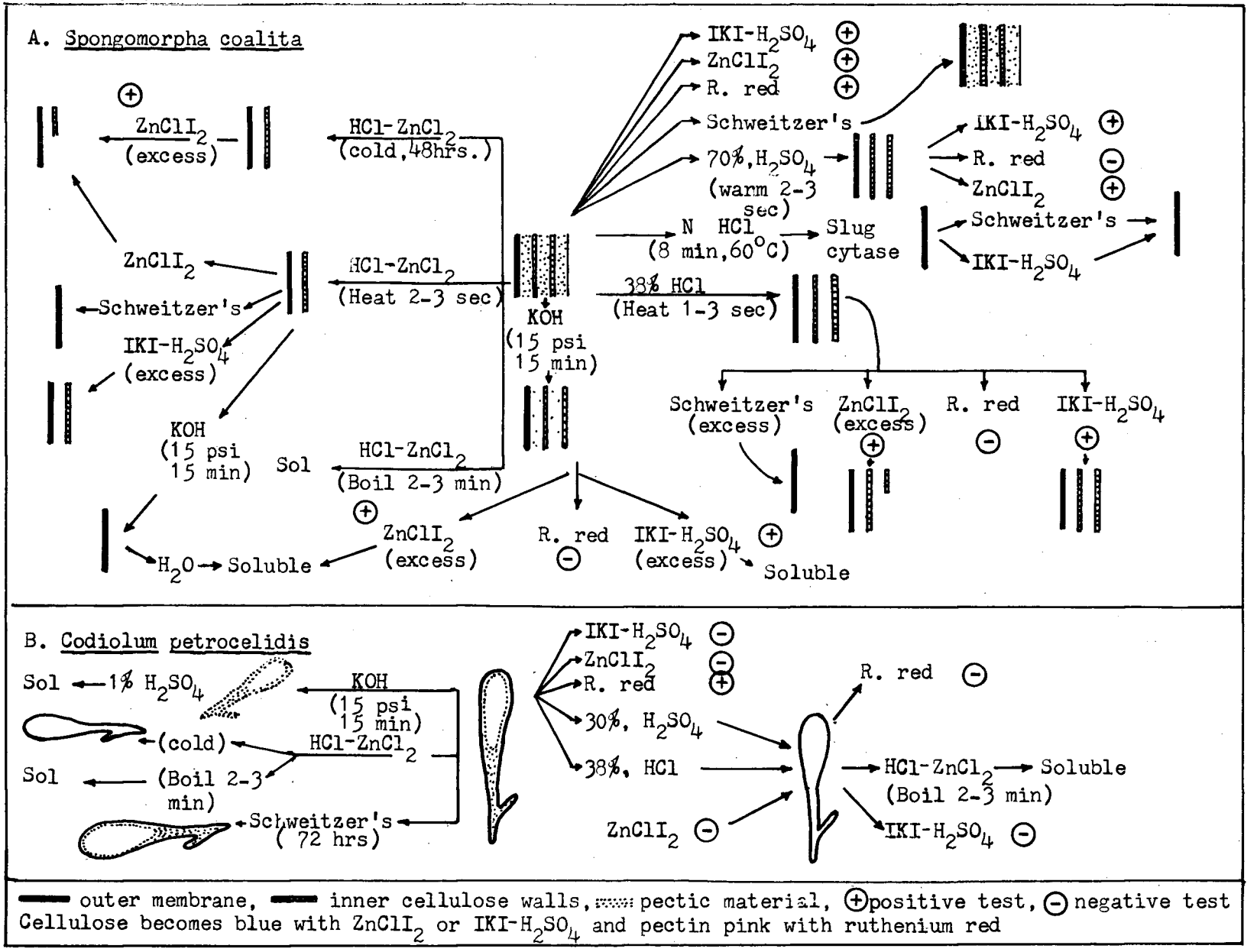

EFFECT OF THERMOPERIOD ON SEXUALITY IN UROSPORA
°F °C
TEMPERATURE
40
30
20
10
100
90
80
70
60
50
0
1
2
3
4
5
6
7
TIME IN HOURS
40 ± 2°C
32 ± 2°C
29.5 ± 2°C
24 ± 2°C
21 ± 2°C
16 ± 2°C
F11 (3)
M2A Dead
URX1
F11 C (10) (14)
M2A Dead
URX1
F11 C (10) G* (15)
M2A Larger nuclei
URX1
F11 C (10)
M2A
URX1
F11 C (10)
M2A
URX1
F11 C (10)
M2A
URX1
F11 C (10) G* (15)
M2A
URX1
CONTROL
BASAL TEMP.
8-10°C
THERMOPERIOD BOX
Culture 10°C
Daily
Thermoperiod
Culture 10°C
7 days
14 days
14 days
F11 - Female clone of U. wormskioldii
M2A - Male clone of U. wormskioldii
URx1 - Unknown clone of U. wormskioldii
(Produced Codiolum cells on two
separate occasions)
* Gametes in only one of the two tubes.
( ) Day of thermoperiod
C - Codiolum
G - Gametes

FIGURE 31 CYTOCHEMICAL TESTS ON THE CELL WALLS OF U. WORMSKIOLDII AND C. GREGARIUM



outer membrane, inner cellulose walls, pectic material, ⊕ positive test, ⊖ negative test
Cellulose becomes blue with $ZnClI_2$ or $IKI-H_2SO_4$ and pectin pink with ruthenium red

FIGURE 32 CYTOCHEMICAL TESTS ON THE CELL WALLS OF S. COALITA AND C. PETROCELIDIS



outer membrane, inner cellulose walls, pectic material, ⊕positive test, ⊖ negative test
Cellulose becomes blue with $ZnClI_2$ or IKI-H_2SO_4 and pectin pink with ruthenium red

FIGURE 33

Urospora wormskioldii

(A - H, field. I, J. culture. All fixed in 3:1)

A, B. Filament under polarized light showing birefringence of walls. A, x 100. B, x 500.

C. Wall layers of two adjacent cells. Ruthenium red. x 500.

D. - F. Walls stained with IKI - H_2SO_4 showing separation of wall layers.

D. Cells showing wall formation between dividing cells. x 500.

E. Cells showing the sheath (sh) clearly separated from the inner wall layers. x 500.

F. Note furrow (arrows) in cell undergoing division. x 500.

G. Filaments boiled in 38% HCl for 3 min showing birefringence under polarized light. x 100.

H. Basal section of filament after autoclaving in 23 M KOH at 15 psi for 15 min. Outer sheath dissolved, inner walls remained, IKI. H_2SO_4. x 500.

I, J. Cultural filaments boiled for a few seconds in 38% HCl followed by digestion with undiluted slug cytase for 4 hrs. Inner walls dissolved, outer sheath remained.

I. Outer sheath showing no birefringence under polarized light. x 300.

J. Cells completely free in outer sheath. x 300.

Scale length, 50 /u

FIGURE 33

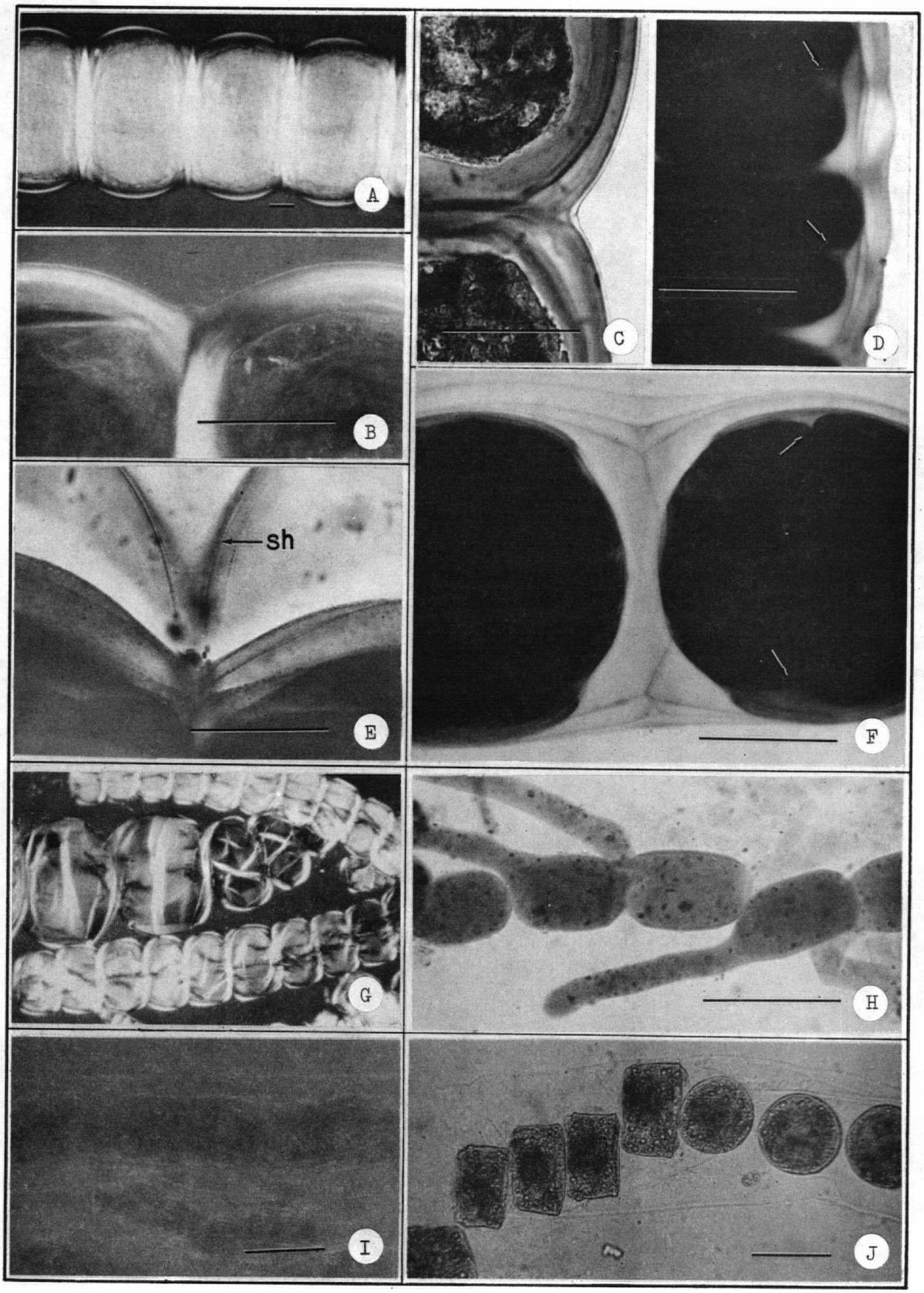


FIGURE 34

A, C - Urospora wormskioldii

B, D - H - Codiolum gregarium

(All fixed in 3:1)

A, C. Autoclaved in 23 M KOH at 15 psi for 15 min and stained with $ZnCl I_2$. Inner layers remained, outer sheath dissolved on autoclaving. Phase. x 100.

B. Untreated cells. x 100.

D. Untreated cells under polarized light showing birefringence in walls, stipe and banded pattern in protoplast. x 200.

E. Boiled in 38% HCl for 2 min. Cells showing protoplast free in outer sheath. Pectic layers of stipe dissolved. Phase. x 500.

F. Boiled in 38% HCl for 2 min. Cells under polarized light showing absence of birefringence in the outer sheath, and presence of a banding pattern in the protoplast of one cell. x 200.

G. Portion of stipe after autoclaving in 23 M KOH at 15 psi for 15 min. Outer sheath dissolved, inner pectic layers remain. x 500.

H. Portion of stipe, untreated and stained with ruthenium red to show the pectic lamellations. x 500.

Scale length, 50 μ

FIGURE 34

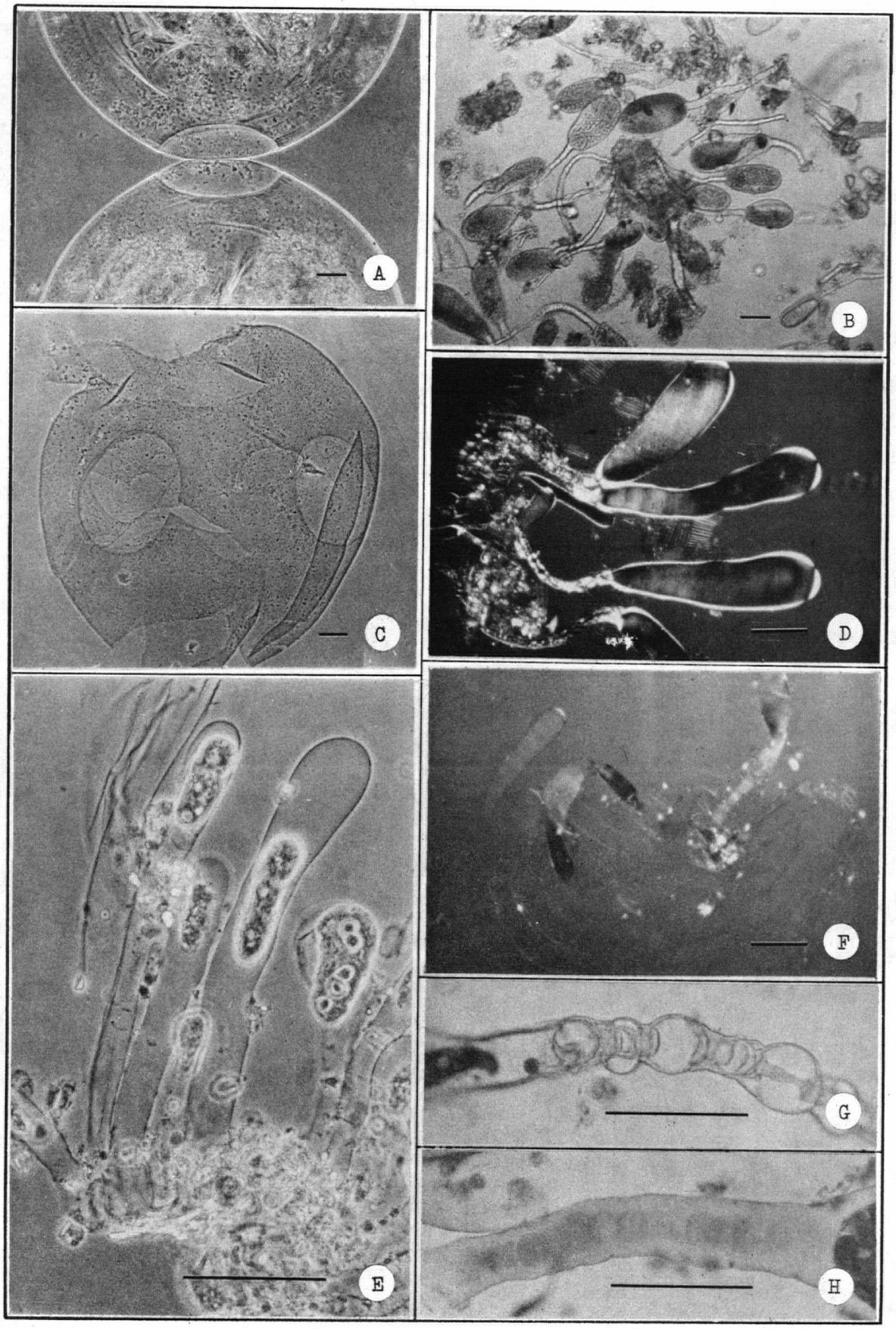


FIGURE 35

A, C, D, F - Spongomorpha coalita

B, E, G - Codiolum petrocelidis

(A, C - G, fixed in 3:1. B, fixed in formalin)

A. Untreated filaments. x 100.

B. One cell of C. petrocelidis oriented stipe down in Petrocelis franciscana. Untreated. Aniline blue. x 300.

C, D. Boiled in 38% HCl for 2-3 min followed with digestion in undiluted slug cytase for 4 hours.

C. Inner walls dissolved, outer sheath remained. x 100.

D. Inner walls dissolved, outer sheath and part of cross-wall (c) left. x 100.

E. Section of stipe. Untreated. Phase. x 500.

F. Heated 2-3 sec. in HCl-$ZnCl_2$ followed with an excess of Schweitzer's reagent, inner walls dissolved, crosswall shadows (c) left. Phase. x 100.

G. Boiled in 38% HCl for 2-3 sec, inner pectic layers dissolved, outer sheath remained. Phase. x 500.

Scale length, 50 /u

FIGURE 35

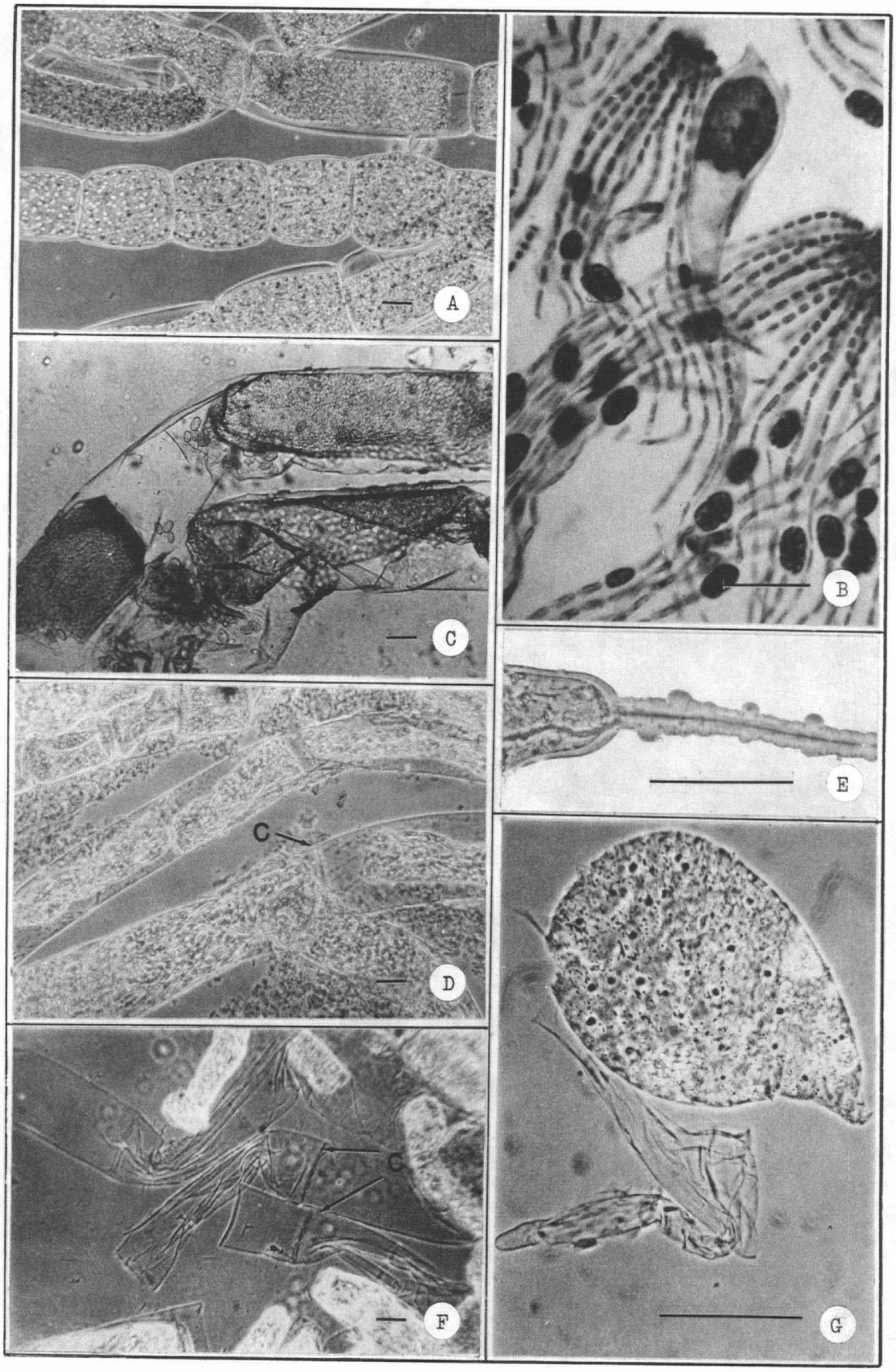


FIGURE 36

A. After Kuckuck, P. (1894, P. 259, Fig. 27) Codiolum petrocelidis n. sp. in Petrocelis hennedyi. Helgoland. A normal cell, and cells with lateral insertions of the stipe on the cell proper.

B. After Printz, H. (1926, Figs. 64 - 72) Codiolum petrocelis Kuckuck in Cruoria pellita showing cells with lateral insertions of the stipe and a lateral appendage on the stipe of one cell.

C. After Kornmann, P. (1961a, p. 196, Fig. 1) Codiolum petrocelidis plants from Petrocelis hennedyi, Helgoland. Several cells having a lateral appendage on the stipe and in one case on the cell proper.

D. After Jónsson, S. (1962, p. 74, Fig. 17, 1a - f) Successive stages in the development of Codiolum petrocelidis in Petrocelidis cruenta. Brittany, France.
(a,b) individuals with their penetration tubes (stipes) oriented towards the exterior of the host.
(c,d) individuals with the first stipe reabsorbed.
(e) individual which has changed its direction of growth and is producing a new stipe at the opposite end of the cell.
(f) a mature individual with a long second stipe and the first formed stipe remaining as an apical thickening.

E. After Jonsson, S. (1958, p. 236, Fig. 11) A stage in the development of Codiolum cells reportedly derived from zoospores of Codiolum petrocelidis, Brittany, France, showing two membranes (septa) in the stipe.

F. After Fan, K. C. (1959, p. 6, Fig. 29, 34)
(29) a stage in the development of a zygote (C. petrocelidis) from S. coalita. Moss Beach, California.
(34) Young cells of C. petrocelidis which developed from zygotes of S. coalita that were allowed to infect Petrocelis in laboratory cultures. The septa are shown here clearly.

G. After Kornmann, P. (1961a, p. 196, Fig. 1). Codiolum petrocelidis plants from Petrocelis hennedyi, Helgoland, showing the septate condition of the stipe.

H. After Hollenberg, G. J. (1958, p. 250, Fig. 1 - 6) showing stages in the germination and growth of zygotes of Spongomorpha coalita. Moss Beach, California.

FIGURE 36

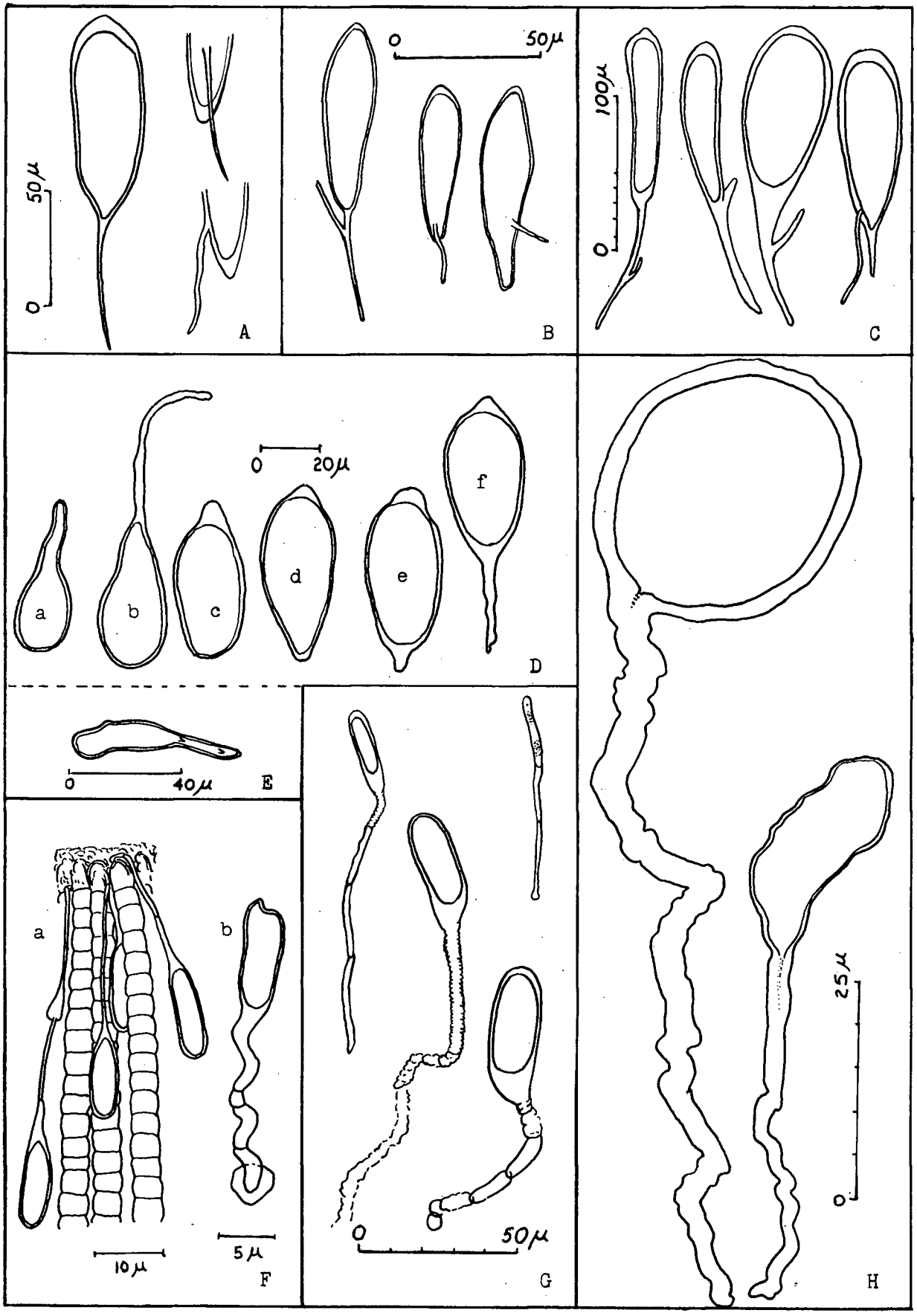

50μ
0
A
0
50μ
B
100μ
0
C
0
20μ
a
b
c
d
e
f
D
0
40μ
E
a
b
10μ
5μ
F
0
50μ
G
25μ
0
H

FIGURE 37

A. Codiolum cell from Urospora clonal culture URX1 showing shallow "U" shaped pectic lamellations in the stipe. x 500.

B - E. Codiolum petrocelidis

B. Cell from Porlier Pass, August 1962, showing first and second formed stipe. Note the opposite direction of the "V"-shaped lamellations taken in the first formed stipe (3). Phase. x 1000. Whole cell, x 500.

C-E. Cells from Deadman's Bay, June 1961. Note the "septa" like structures (s) distal to the clava. Safranin. x 500.

D. Cells growing with stipe directed up within Petrocelis franciscana. Safranin. x 500.

Scale length, 50 /u

FIGURE 37

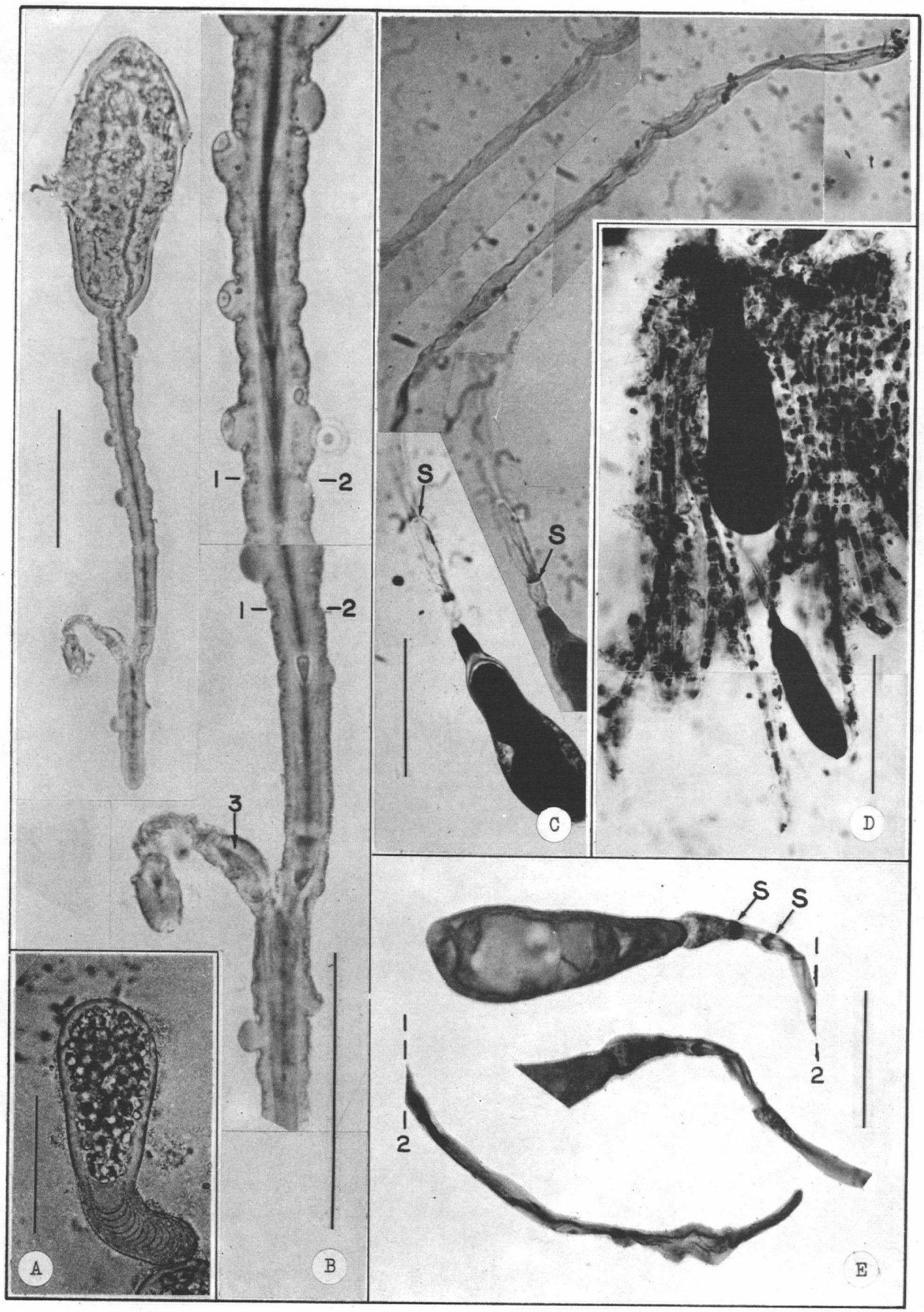


FIGURE 38

Codiolum petrocelidis in Petrocelis franciscana

A, B. C. petrocelidis from Porlier Pass, August 1962. 3:1 fix. Aniline blue.

(1) Cell with stipe at both ends.

(2) Cell with stipe oriented down in the host.

(3) Cell with stipe oriented up, and protoplast just starting to change its direction of growth. Lamellations (1) of new stipe seen at lower end. A, x 100. B, x 600.

Scale length, 100 /u

FIGURE 38

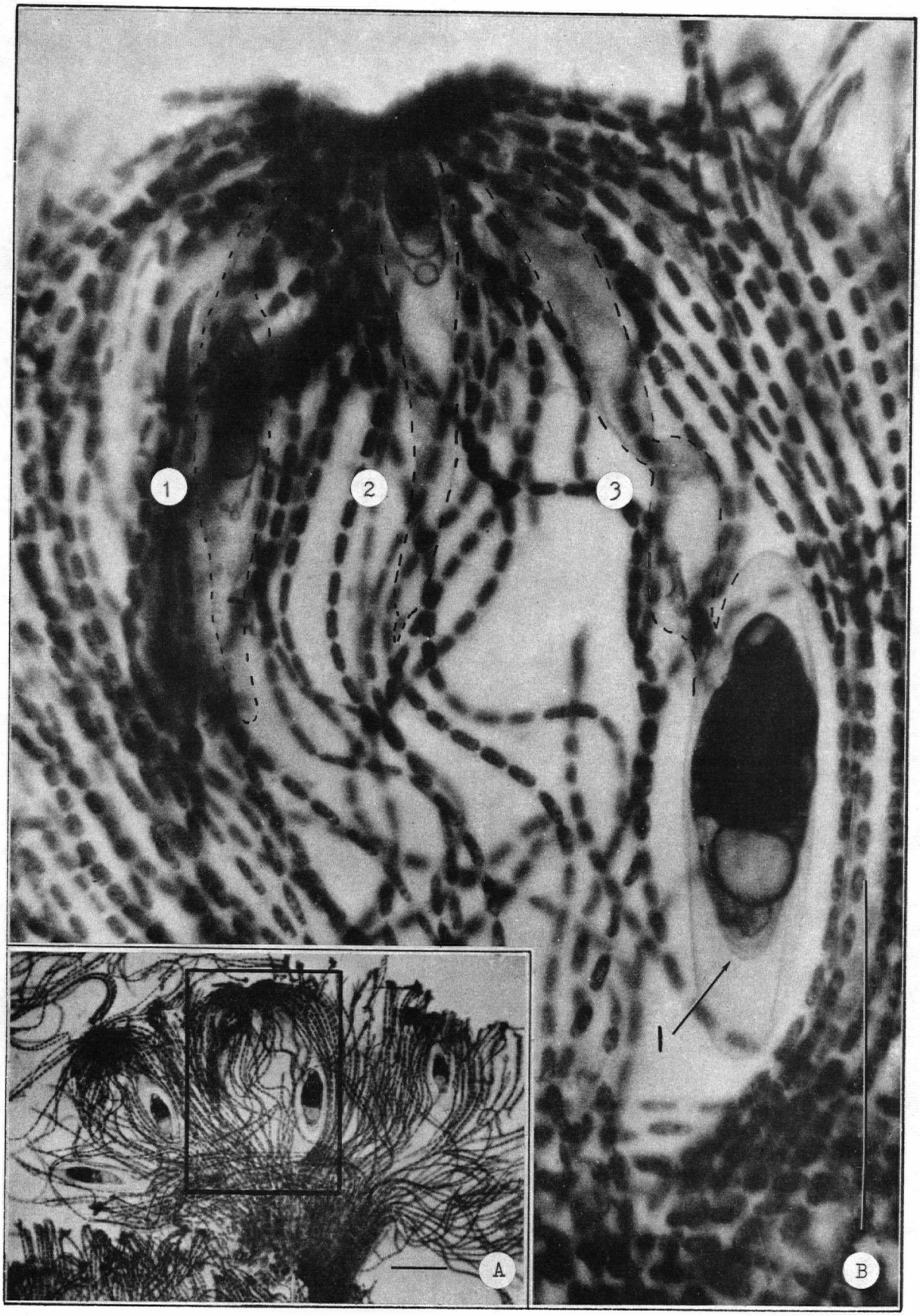


FIGURE 39

A, B, D - F. *Codiolum petrocelidis* in *Petrocelis franciscana*
C. *Spongomorpha coalita*

A. Two cells of *C. petrocelidis* from Porlier Pass, with stipes oriented up. 3:1 fix. Aniline blue. Phase. x 500.

B. A cell of *C. petrocelidis* from Porlier Pass, with change in direction of stipe growth. 3:1 fix. Aniline blue. Phase. x 500.

C. *Spongomorpha coalita* from Mussel Point, Monterey, California, June 1960. A portion of a fertile filament showing an empty gametangium with an operculum (o). Dried herbarium specimen. Phase. x 200.

D. A cell of *C. petrocelidis* from Point No Point, December 1963, showing a prominent apical cap. Formalin. Aniline blue. x 200.

E, F. Cells of *C. petrocelidis* from Point No Point, December 1963, oriented stipe down in fertile *Petrocelis franciscana*. Formalin. Aniline blue. x 100.

Scale length, 50 μ

FIGURE 39

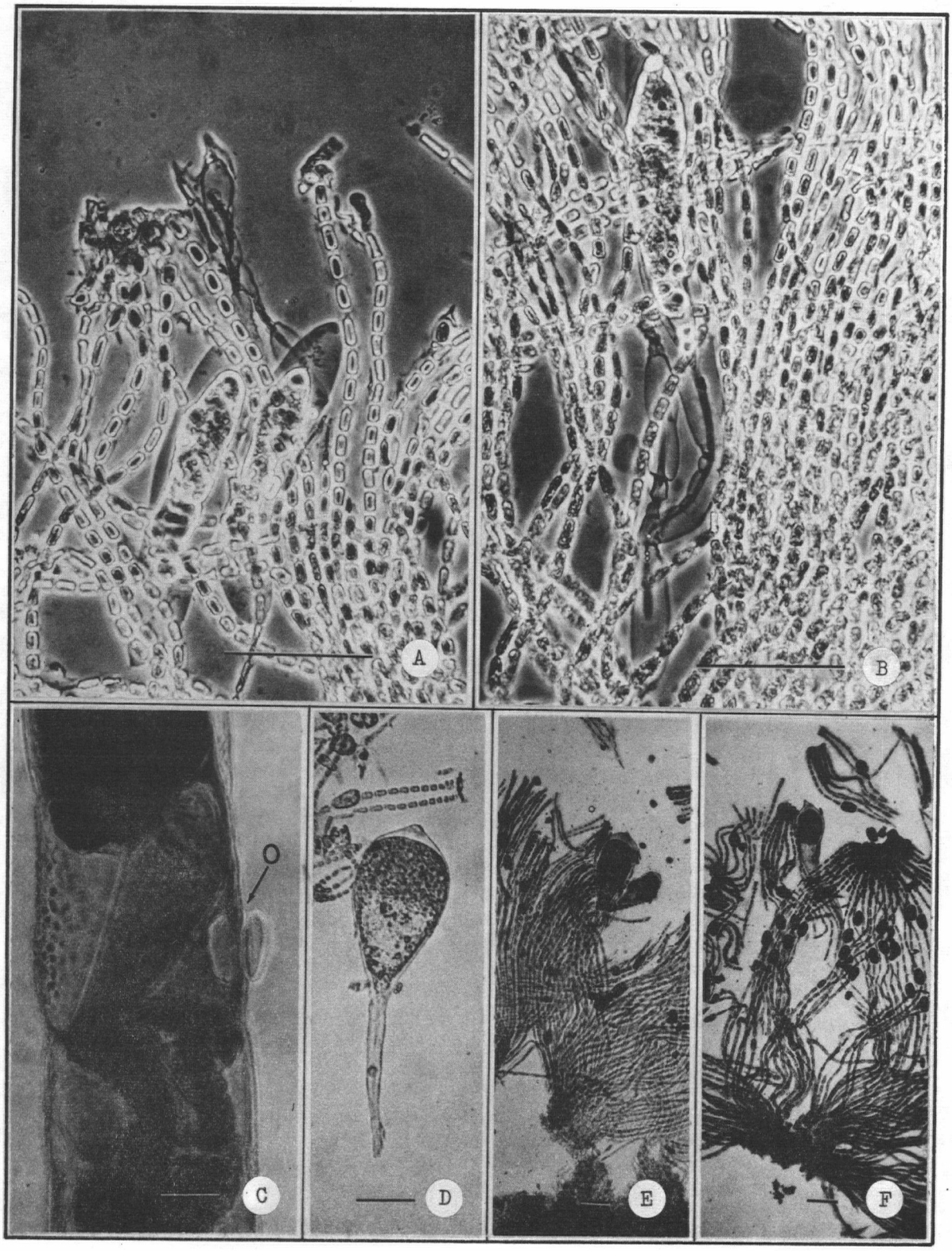


FIGURE 40

Codiolum petrocelidis

(A - D, fixed in 3:1. E, fixed in formalin)

A - D. From Porlier Pass. Stages in formation of second stipe. Cell A, B, D, are early stages and cell C, a later stage in development. Phase. x 500.

E. From Point No Point. Cell with first stipe broken off at arrow. Phase. x 200.

Scale length equals 50 μ

FIGURE 40