Bangia vermicularis Harv.

in the Vicinity of Charleston, Oregon

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During the 1971 summer session at the Oregon Institute of Marine Biology (OIMB), Charleston, Oregon, a study of the alga Bangia was undertaken, as a part of the requirement for the 433 Algae class. The dual aims of the study were, firstly, to acquaint the student with techniques of collection, culture, study, and literature search on a particular alga; and secondly, to add to the cumulative records on this alga. The first objective was accomplished. Due to this student's interest in the techniques of making permanent slides of algae, a small contribution may have been made to the second, in the form of microscope slides prepared and deposited with Dr. Byron Lippert, of Portland State University, plus records kept as reported below.

Beginning with a complete ignorance of even the appearance of the plant, the writer employed the following techniques in the study: identification, field recognition, search for and reading of material on morphology and life cycles, repeated collecting, microscopic examination of each collection, making of permanent whole mount microscope slides, and attempts to culture spores.

The plant here studied is given by Smith, Doty and Dawson as Bangia vermicularis Harv., in the family Bangia-

ceae, order Bangiales, subclass Bangioideae, class Bangiophycidae, and the division Rhodophyta. The genus was founded in 1819 by Lyngbye in honor of his teacher, Niels Hofmann Bang, a Danish botanist of Copenhagen.

Habitat and Distribution:

Bangia vermicularis Harv. is a small uniseriate or multiseriate filamentous alga, resembling blackish hair, found, in the vicinity of OIMB, on creosote-impregnated dock pilings or the vertical faces or tops of rocks near the high intertidal level.

In the younger uniseriate filaments, cells are cylindric, about 15u by 15u, but as the filaments age, intercalary transverse, longitudinal or oblique divisions produce cells much wider than high and of various shapes. The cell contains a single, centrally located, stellate chloroplast containing one pyrenoid. A colorless matrix or wall encloses the cells. The filaments are secured to the substrate by rhizoids which originate from the lower cells and grow down intramatrically, to emerge at the bottom and form a holdfast. Members of the Bangiophycidae were formerly thought not to have pit connections between cells, but recent work is questioning this.

Life cycle:

Regarding the life cycle of Bangia, there is far from complete certainty and unanimity among various sources. Tilden (1937) states confidently that reproduction is by gonidia and by fusion of sperms and eggs borne on male and

female plants, and portrays these structures with excellent drawings. She says further that all cells, except basal cells, of the male plant divide to form antheridia, 64-128 per cell. In the female plant, each vegetative cell becomes a carpogonium. Meiosis is believed to occur immediately following fertilization, since the fertile egg divides at once into 8 spores.

Smith (1969) says spermatangia are formed by division and redivision of vegetative cells, and carpogonia with short trichogynes are formed by direct metamorphosis of vegetative cells, the zygote dividing to form 4 or 8 carpospores. Asexual reproduction is by division of vegetative cells into 2, 4, or more cells, each of which becomes a spore.

Doty (1947) reports that "cystocarpic material" had been collected at Rogue River Reef in July. In fertile segments of uniseriate filaments, each cell had undergone two divisions with each of four resultant cells putting forth a trichogyne.

Bold writes that in asexual reproduction, protoplasts of both uniseriate and pluriseriate filaments are liberated as monospores which may be amoeboid. (This correlates best with the writer's observations). "Sexual reproduction, although reported for Bangia, is imperfectly known."

Neither Drew (1952) nor the team of Richardson and Dixon (1968) observed sexual reproduction in cultured material, although Drew described spermatia. Drew also saw formation of monospores, some amoeboid, and subsequent bipolar germination to a Conchocelis phase, similar to that found in

Porphyra. Richardson and Dixon cultured the "complete life cycle" of Bangia from Conchocelis stage to Bangia and back again, by inducing different types of germination in response to hours of daylight. Over 12 hours of daylight per 24 hours produced unipolar germination to the Conchocelis stage; less than 12 hours of daylight produced bipolar germination to Bangia; at 12/12, both types of germination occurred.

At temperatures of 5-15°C., the Conchocelis stage produced either plantlets or fertile cell rows, but at 20°C., formation of the latter was completely inhibited. They also describe the Conchocelis chloroplast as parietal. They detected neither cell fusion nor meiosis.

Actual Procedure of the Study:

First readings were done in general texts and manuals on hand at the station: Dawson, Doty, Bold, Johansen, Smith, Prescott, and Tilden, the last seeming the most useful source at the beginning. Later, one search of recent literature was made at the University of Oregon Science Library at Eugene. The most helpful article found was by Drew (1952).

A plan for the study devised early in the work, was to collect regularly from the boat basin at low tide and high tide, two or three times a week, to observe in sea water under the microscope, watching for reproductive structures and attempting to identify which ones, and to preserve material from each collection, some in 10% formalin in sea water, some on permanent slides. The design of the plan was repeat-

edly altered, due to what may best be termed Unforeseen Contingencies.

However, eighteen collections were made, 14 of them from the Charleston boat basin, 2 from Area 9, North Cove, Cape Arago, and 2 from Area 4, North Cove. All of the boat basin specimens were cut from creosote-impregnated wooden pilings, in areas of much-polluted water. Nearly all were taken at low tide. The four from North Cove were scraped from rocks at low tide, in areas of relatively clean water.

Records were kept of date and time of collection, correlated with tide information, day length, locality, habitat, observations, and final disposition of the material. See Table 1.

Collections were placed immediately in sea water and examined as soon as possible, first, gross examination in a watch glass under a dissecting microscope, then on a microscope slide in sea water, under a coverslip, at 100X and 440X. Notes, not complete for all specimens, were kept of these observations.

Further, samples of nearly all collections were preserved on slides, by the fast green/acetic acid method (see Appendix I) or in CMC-9 or CMC-10 (see also Appendix I). This technique had the two-fold value of preserving voucher specimens, plus keeping the material while the author learned what she should be looking for.

Material from nearly all collections was preserved in 10% formalin in sea water. These samples are at Eastern

Michigan University, Ypsilanti, Michigan.

Structures positively identified were: uniseriate and multiseriate filaments; intramatrical rhizoids; monospores (being emitted); spore germination *in situ*; and stellate chloroplasts.

Structures tentatively identified as carpogonia were seen on slide O-51-4, and spermatia formation on O-99-3. Clumps of small filaments attached to older ones appeared to be the result of recent spore germination. No evidence of a *Conchocelis* stage similar to that of *Porphyra* was seen, other than a dubious observation on O-35-1, or in the photographs and sketches of germination (see figure 3 and sketches).

A differential staining was observed with the fast green technique, which would warrant further investigation, some cells staining green, some pink, some bright purple-blue, some greenish-brown. This might be a function of the age of the cell, but also might reflect male and female structures, or physiological state. Actively dividing cells on O-51-4 stained pink. This differentiation faded with time, being most noticeable when first done. Possible correlation of staining results with cell physiology is indicated by Tripodi (1968).

Collections from North Cove seemed coarser than those from the boat basin, but under the microscope this appeared to be due to numerous epiphytes. why?

Several attempts were made to culture spores. Although there was copious spore discharge from filaments, and spore

germination was observed, sketched, and photographed, no culture continued successful, due chiefly to uncontrolled growth conditions. Experience indicates the culture methods given in Appendix III to be the most useful. See Appendix II for data on the photographs, Figures 1, 2, 3, and 4.

In addition, fresh material was brought back to Eastern Michigan University in the hope it could be cultured there, under varying photoperiod lengths. Technical difficulties prevented this, but the material remained alive in sea water solution (brought from Oregon), with air bubbled through, under fluorescent lights, for two months.

Suggestions for further study:

Practical suggestions to a student attempting a similar study, based on the writer's successes and failures, would be:

1. From the beginning, label everything immediately--slides, vials, herbarium sheets.
2. Set up a looseleaf notebook at the start of the study, in which to record everything concerned with the project--reading notes, collection notes, observations, and records of preservation. Have a separate page for each collection.
3. Read about life cycle and morphology in standard references.
4. Search for and read recent publications on the plant. Especially recommended are the three articles cited here: Drew (1952); Richardson and Dixon (1968); and Hollenberg (1958).
5. From the beginning, search for, observe, and preserve specimens while learning about the above.

As with any investigation, more questions were raised than answered.

1. What is the meaning of the differential staining produced by the fast green/acetic acid method?

2. Bangia is frequently found in proximity to the barnacle Balanus. Could a Conchocelis stage be associated with that organism?

3. Spore germination studies similar to those of Drew should be carried out, as well as carefully controlled experiments as to the correlation between day length and type of spore production and germination. Would an optimum time to study germination be at the equinoxes, when both types might be observed in wild material?

4. If, as Richardson and Dixon state, only germination to the Conchocelis phase occurs at longer day lengths, why were obviously very young bipolar filaments found in the boat basin collections during July?

5. Why is there a differently shaped chloroplast in the Conchocelis stage, as reported by Richardson and Dixon?

6. Finally, does Bangia represent a plant of sufficiently undifferentiated morphology to retain great plasticity of response to changing conditions, having, as it does, simple and multiseriate filaments, a prostrate creeping filament, two types of germination (at least), and even an amoeboid stage?

7. Occurrence of epiphytes on North Cove Material & their absence in the boat basin.

Summary:

In retrospect, the study proved valuable in the education of this student in research, collection, preservation and culture techniques. Although only simpler and more common morphological structures were recognized by the writer during the initial work, it is hoped that later, more intensive examination of the slides made may reveal sexual structures.

It should be recognized that these slides were made from wild collections and are much contaminated by other organisms whose appearance is subject to misinterpretation.

Although this study was of limited scope, perhaps a later investigator can build on the bibliography, techniques, permanent slides, and recommendations to make a contribution to the records on Bangia vermicularis.

COLLECTION Coll. no.	Place	Date	Fast grn/ acet.	CMC9	Other	10% Form.	Time of Coll.	Time & height of tide		Day length	Height above water
0-31	CBB I	6/27/71	1	--	--	X	10:45 AM	L 10:06 AM	-0.4	15h 28min	4'
0-32	CBB II	6/28/71	2	--	--	X	8:30 AM	L 10:42 AM	0.2	" "	6'
0-33	CBBII	6/28/71	1	--	--	X	8:30 AM	L 10:42 AM	0.2	" "	3'
0-34	CBB I	6/30/71	--	--	--	--	11:15 AM	L 12:30 PM	2.2	" "	2'
0-35	CBB II	6/30/71	2	--	--	X	11:15 AM	L 12:30 PM	2.2	" "	2'
0-46	CBB I	7/2/71	4	--	--	X	1:00 PM	L 1:30 PM	2.4	" "	1'
0-47	CBB II	7/2/71	2* + 1	1	--	X	1:00 PM	L 1:30 PM	2.4	" "	1'
0-50	CBB I	7/5/71	4	--	--	X	12:30 PM	H 12:12 PM	4.1	" "	-1'
0-51	CBB II	7/5/71	3	--	--	X	12:30 PM	H 12:12 PM	4.1	" "	-3"
0-75	Area 4 N Cove	7/10/71	4	4	--	X	9:00 AM	L 8:30 AM	-1.4	15h 17min	top of rock
0-76	Area 4 N Cove	7/11/71	--	6	--	X	9:00 AM	L 9:06 AM	-1.1	" "	top of rock
--	CBB	7/19/71	--	--	--	--	7:10 PM	H 9:18 PM	7.0!	" "	not visible
0-79	CBB I	7/20/71	4	--	3 CMC10	X	5:00 PM	L 5:06 PM	3.1	" "	1'
0-80	CBB II	7/20/71	4	--	4 CMC10	X	5:00 PM	L 5:06 PM	3.1	" "	1'
0-86	Area 9 N Cove	7/20/71	--	--	--	X	6:30 AM	L 5:48 AM	-1.2	15h 02min	top of rock

Table 1

COLLECTION Coll. no.	Place	Date	Fast grn/ acet.	CMC9	Other	10% Form.	Time of Coll.	Time & height of tide			Day length	Height above water
0-98	CBB I	7/22/71	4	--	4 CMC10	X	7:00 PM	L	6:48 PM	2.8	14h 57min	--
0-99	Area 9 N Cove	7/22/71	4	4	--	X	7:30 AM	L	7:12 AM	-1.2	" "	--
0-133	CBB I	7/26/71	4	--	4 CMC10	X	9:45 AM	L	9:24 AM	0.2	14h 41min	3.5'
0-138	CBB I	7/29/71	--	--	--	X	12:20 PM	L	11:00 AM	1.2	" "	1'

Table 1 (cont)

Abbreviations:

CBB I = Charleston Boat Basin, piling beside floating dock, D27-D28

CBB II = Charleston Boat Basin, 2nd piling in from far end of floating dock.

Area 9, N Cove = North Cove, Cape Arago, Coos Co., Oregon

Area 4, N Cove = same

H = high tide

L = low tide

* = hematoxylin in sea water used for stain

Photographs of germinating Bangia spores (0-99) at 800X.
(See Appendix II for data.)

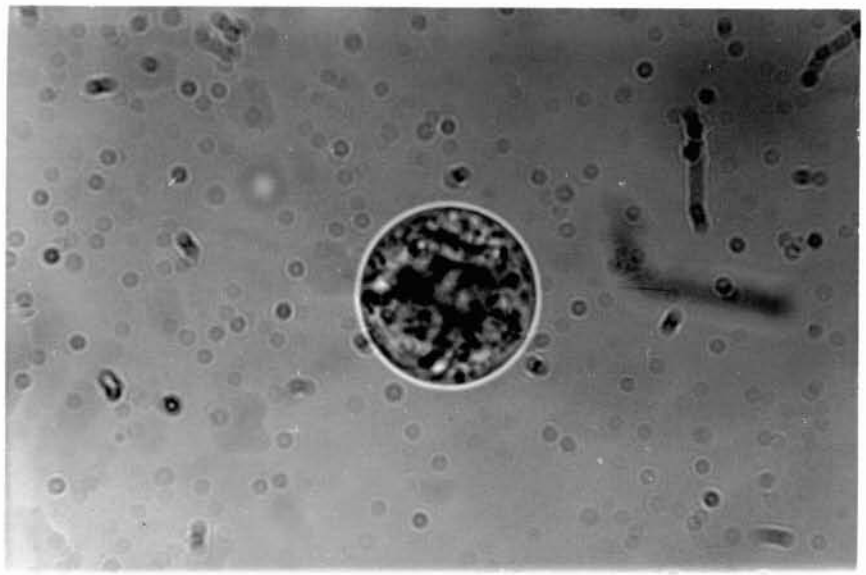


Fig. 1.
Ungerminated spore, showing stellate chloroplast.

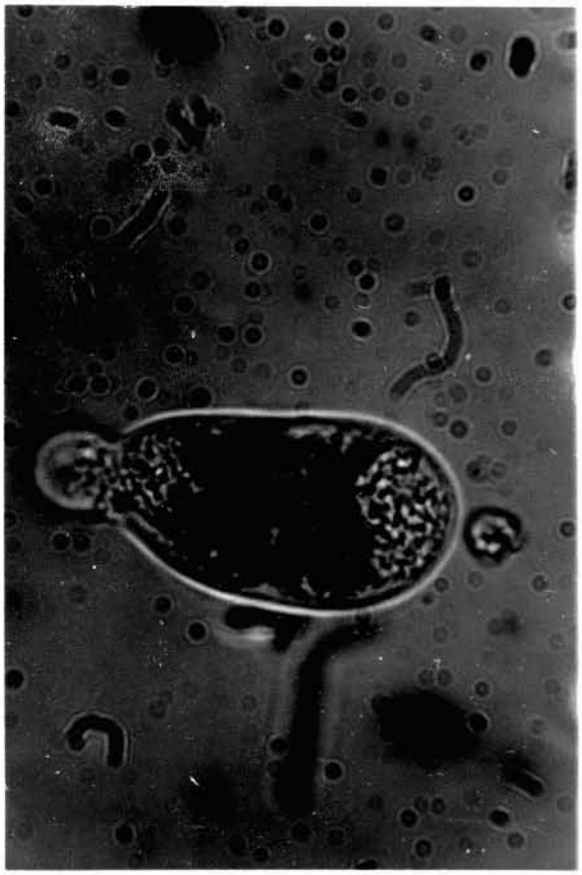


Fig. 2.
Enlarged spore, germinating, chloroplast dividing.

Fig. 3.
Two spores, germinating, one showing elongation, and division of chloroplast.

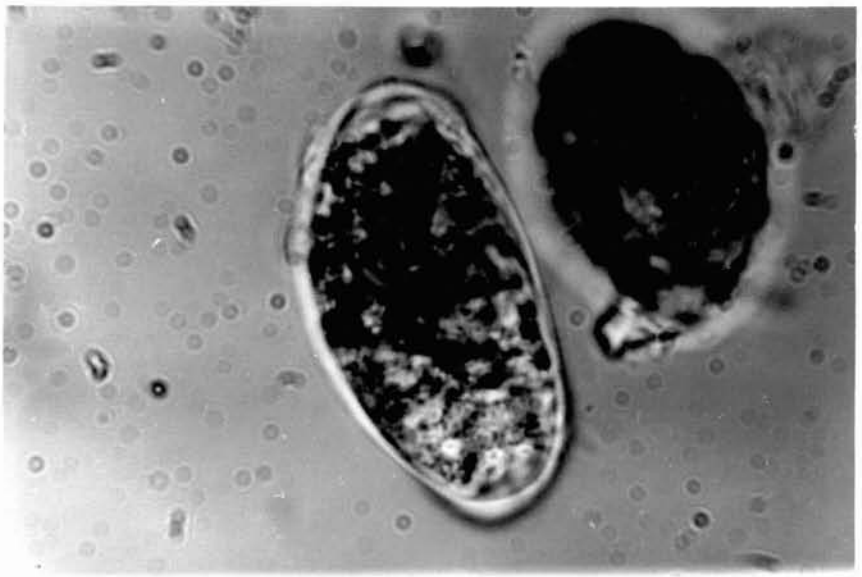
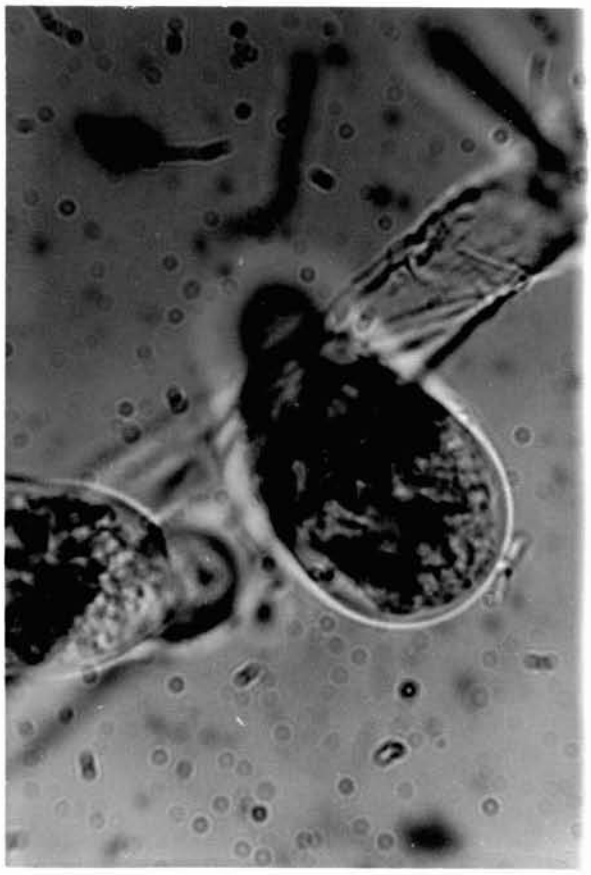
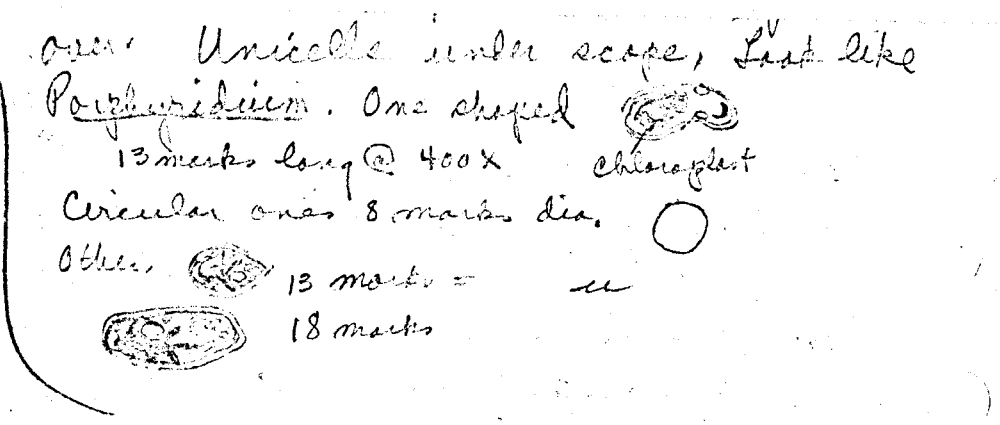
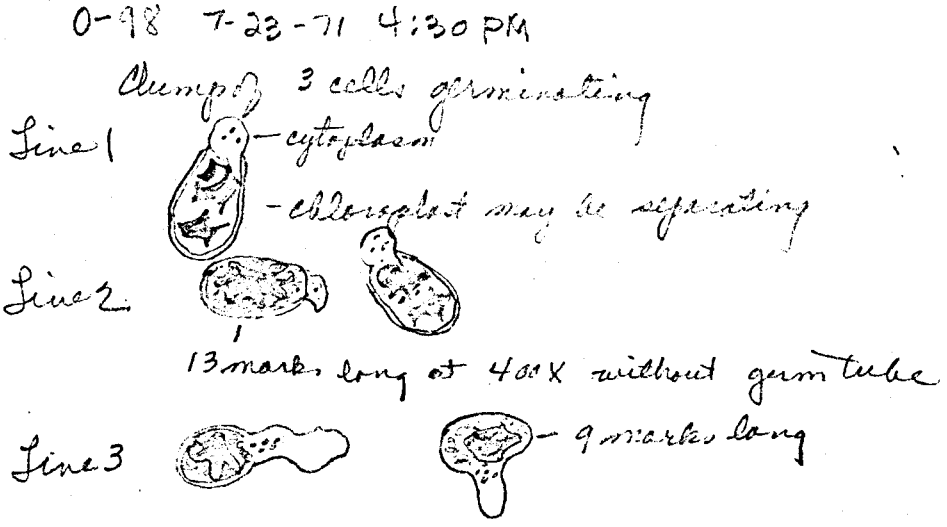


Fig. 4.
Two spores, germinating, with remnant of empty filament.



Sketches of germinating spores of Bangia (O-99), taken from laboratory notes. There is some suggestion here of germination to the creeping filament stage. It was considered better to xerox the sketches, instead of converting them to ink drawings, to keep the immediacy of the original observations.





Appendix I

Quick permanent mount for microscopic algae, adapted from Brandham (1970)

Materials:

Mayer's egg albumin	100% ethyl alcohol (EtOH)
Fast Green stain, dry powder	microscope slides
Euparol (Diaphane)	cover slips
Euparol Essence	95% EtOH
Glacial acetic acid	

Mix the following:

Euparol essence--equal parts Euparol and 100% EtOH
1:6 Acetic-alcohol (1 part glacial acetic: 6 parts 100% EtOH)
1:3 Acetic-alcohol
1:1 Acetic-alcohol
2:1 Acetic-alcohol
1:1 100% EtOH: Euparol Essence
0.15% Fast Green in glacial acetic (0.15 gm. Fast Green/100 ml.)
1:6 Acetic-alcohol diluted 1:1 with sea water

Line up solutions:

1. Mayer's albumin - refrigerate when not in use. (Note: Haupt's affixative equally as good)
2. Algal suspension
3. 1:6 Acetic-alcohol diluted 1:1 with sea water
4. 1:6 Acetic-alcohol
5. 1:3 Acetic-alcohol
6. 0.15% Fast green in acetic
7. Glacial acetic
8. 2:1 Acetic-alcohol
9. 1:1 Acetic-alcohol
10. 1:3 Acetic-alcohol
11. 100% EtOH I
12. 100% EtOH II
13. 1:1 100% EtOH:Euparol Essence
14. Euparol Essence
15. Euparol
16. Cover slips stored in 95-100% EtOH

Clock or watch with sweep second hand
Flat dish to catch drips
Newspapers to protect work surface
Paper towels to protect dissecting scope

Steps in slide making:

Caution!! Work on newspapers and protect clothing and dissecting scope. Fast green stains everything, and glacial acetic acid burns!

1. Thoroughly clean slide with soap and water, then with 95% EtOH.
2. Write code number on left end of slide with wax pencil.
3. Using finger, coat slide thinly with Mayer's albumin affixative.
4. Pipette on 1 drop of algal culture.

5. Using scraps of paper towel, keeping slide horizontal, working under dissecting scope, carefully draw off excess water until algae is drawn down into affixative (Mayer's albumin). Do not let dry out completely, now or at any time during the series.
6. Fix with 1-2 drops of 1:6 acetic-alcohol diluted 1:1 with sea water. (Note: this step is necessary for marine algae only).
7. Leave above on for 2 min.
8. Gently drop on 1-2 drops of 1:6 acetic-alcohol for 2 min.
9. 1-3 drops of 1:3 acetic-alcohol for 2 min. (Note: now and at any time, blot off any excess fluid with scraps of paper towel.
10. 1-2 drops of Fast Green for 1 min. only.
11. Wash out excess stain with 1-3 drops glacial acetic for 2 min.
12. 1-3 drops 2:1 acetic-alcohol for 1 min.
13. 1-3 drops 1:1 acetic-alcohol for 1 min.
14. 1-3 drops 1:3 acetic-alcohol for 1 min.
15. 1-3 drops 100% EtOH for 1 min.
16. 1-3 drops 100% EtOH/Euparol Essence for 2 min. (This is the beginning of the infiltration of the tissue with Euparol.)
17. 1-3 drops Euparol Essence for 1 min.
18. 1 drop Euparol.
19. Carefully lower coverslip, which has been stored in 95-100% EtOH, from one side, with forceps, down over Euparol.
20. Dry in horizontal position, away from dust, for a week or more. Store in horizontal position. Clean with 100% EtOH.

Labeling:

Print a microscope label, using India ink and a very fine pen point. Include the following information:

Code or catalog number
Name of collector
Date of collection
Name of organism
Stain used
Mountant used (Euparol)

Bangia vermicularis Lvg. 0-31

Fast green
Euparol
R. Alford VI/30/71

CMC-10, CMC-9: artificial mounting media obtainable from Turtox, CCM: General Biological Supply House, Inc., 8200 S. Hoyne Avenue, Chicago, Illinois, 60620.

CMC-9 seems to be superior for this purpose.

Appendix II

Subculture of Bangia spores on a microscope slide, within a square of vaseline, covered with coverslip. Observed and photographed under the Zeiss research microscope at an effective magnification of 400X (eyepiece = 3X; objective = 100X; camera factor = 0.5), using 35mm. black-and-white film. After enlargement and printing, the magnification was 800X.

Appendix III

Culture medium (Schreiber, 1931) as given in Johansen (1940) for the Phaeophyta, but seemingly adaptable for the Rhodophyta.

Sodium nitrate	0.1 g.
Dibasic sodium phosphate	0.02 g.
Distilled H_2O	50.0 cc.
Sea water	to 1 liter

Autoclave at 15 pounds pressure for about 20 minutes. May be solidified with 1.5% agar if desired to raise germlings on such a medium.

Johansen recommends stacking slides log-cabin-wise in clean, covered culture dishes, flooding with medium, and introducing bits of material to be cultured. Hollenberg (1958) suggests the use of broken coverslip pieces, which can easily be removed to fresh culture dishes for subculturing. From this writer's experience, the second method is preferable.

For further suggestions on both culture media and techniques, see Starr (1964), Prescott (1968), Johansen (1940), and Hollenberg (1958).

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