Developmental studies on cultured endolithic conchocelis (Rhodophyta)

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Introduction

The discovery by Drew (1954) that *Conchocelis rosea* Batters was not a discrete species of rhodophyte, but the microscopic, endolithic phase in the life history of members of the Bangiaceae (Rhodophyta), provided a new basis for experimental research in this group. Reviews of the life history are presented in Conway & Cole (1977), Cole & Conway (1980) and Campbell (1980). The taxonomy of the bangiacean rhodophytes today is based entirely on features of the macroscopic thallus (see Conway *et al.* 1975).

Krishnamurthy (1969) introduced comparative study in culture of the conchocelis of different bangiacean species, growing them from carpospores shed by taxonomically identified macroscopic thalli collected from nature. He did not use a mineral substrate, so the cultures grew as free-living, rather than endolithic conchocelis. Conway & Cole (1977) evaluated the free-living conchocelis of a large number of *Porphyra* species using light microscopy, and scanning electron microscopy of critical point dried specimens. Their conchocelis was also cultured from the carpospores of identified macrothalli. Their comparative study concluded that 'the conchocelis phase of *Porphyra* and *Bangia* are worthy of fuller recognition than has yet been accorded in any general (taxonomic) consideration of the Bangiaceae'.

Most experimental studies involving conchocelis have been done on free-living rather than endolithically growing cultures, because the shell substrate was perceived as a hindrance to preparation and/or observation of the conchocelis. However, Campbell (1980) demonstrated that the boreholes made by endolithic conchocelis conform closely to the surface morphology of the organism. The preparation of resin casts of the borings of endolithic microorganisms for study by scanning electron microscopy (Golubic *et al.* 1970) allows replication of the borehole (i.e. organismal) morphology with sub-micron level fidelity, including preservation of its three-dimensional (3-D) orientation within the substrate. The traditional approach of studying free-living, cultured conchocelis involves mounting it on microscope slides with cover slips. This destroys the 3-D relationships of the various structures, thereby removing one parameter from taxonomic consideration.

The objective of the current investigation was to determine whether the conchocelis cultured from carpospores of eight bangiacean rhodophyte species and maintained under standardized growth conditions, have characteristically different morphologies when grown endolithically. Our long-range goal is to see whether the endolithic conchocelis of different bangiacean species are sufficiently characteristic to make taxonomic identifications in the laboratory and in the field based on borehole morphology alone. If successful, the results would enable the study of the natural distribution and ecological requirements of the conchocelis of different bangiacean species, independent of the occurrence of the macroscopic phase.

Materials and methods

Shell chips removed from the inner surface of *Macoma* shells were carefully examined under a

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Table 1. Conchocelis width, its variability, and the length of branch axes in μm. Length of branch axes (i.e., branch to branch, and branch to tip of filament) is used as a comparative measure of branching frequency.

Species	Structure	Widest point			Narrowest point			Difference			B-B length (Branch to Branch)			B-T length (Branch to Tip)			Combined B-B & B-T length		
		n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD
P. abbottae	Conchosporangial branches	108	11.6	3.6	107	8.6	3.1	96	2.9	2.4	13	24	16	82	39	21	95	36	20
	Vegetative filaments (excluding swellings)	177	4.9	2.3	179	2.9	1.2	177	2.0	1.9	48	27	16	67	29	30	115	28	25
	Swellings	none noted																	
P. torta	Conchosporangial branches	50	9.4	1.3	50	8.2	1.6	50	1.2	1.2	20	12	4	45	29	14	65	23	14
	Vegetative filaments (excluding swellings)	51	3.5	1.3	51	2.4	0.9	51	1.1	1.2									
	Vegetative filaments (including swellings)	42	3.1	1.0	42	2.3	0.6	42	0.8	0.9	13	23	14	7	28	21	20	25	16
	Swellings	13	6.0	1.3															
P. nereocystis	Conchosporangial branches	15	20.3	5.0	15	13.7	2.0	15	7.3	3.4	5	75	12	10	85	44	15	81	36
	Vegetative filaments (excluding swellings)	32	2.8	1.9	32	1.9	0.6	32	1.0	0.8	not measurable in micrographs: >100 μm average length between branches								
	Vegetative filaments (including swellings)	43	3.8	2.0	43	1.9	0.5	43	1.9	1.9									
	Swellings	11	6.8	1.2															
P. schizophylla	Conchosporangial branch (?)	4	22.5	2.1	4	6.3	1.0	4	16.3	1.9	not measurable in sample micrographs studied								
	Vegetative filaments (excluding beaded filaments)	100	10.3	2.7	100	5.4	1.5	100	4.9	2.8									
	Vegetative filaments (including beaded filaments)	144	9.8	2.8	144	5.3	1.4	141	4.6	2.8	25	37	14	55	34	26	80	36	22
	Beaded filaments alone	42	8.6	2.8	42	4.9	1.1	42	3.6	2.6									
P. variegata	Vegetative filaments	110	4.7	1.6	110	2.8	0.9	110	1.9	1.3	42	15	9	54	16	12	96	15	11
P. fucicola	Vegetative filaments	76	4.8	1.7	76	2.8	2.8	76	2.2	1.6	71	16	10	88	20	15	159	18	13
P. perforata	Vegetative filaments	116	4.8	0.9	116	2.5	0.9	116	2.3	1.5	58	13	12	84	18	11	142	16	11
B. atropurpurea	Vegetative filaments	30	5.8	1.7	30	2.7	1.0	30	3.0	1.7	20	16	7	23	22	10	43	19	10

high-power dissecting microscope to rule out the presence of pre-existing microborings. Both the shell and experimental calcite spar, freshly cleaved for the purpose, were autoclaved separately from the medium, prior to introduction into culture dishes.

Shells of *Macoma, Clinocardium, Protothaca, Saxidomus, Mytilus,* and *Unio* were subjected to acid dissolution with 3% HCl, and the consistency and amount of organic residue was noted.

The following species of conchocelis were grown in Erdschreiber medium (McBride & Cole, 1969) under cool-white, fluorescent lights (at 250 lux or ca 6 $\mu Em^{-2}s^{-1}$ at 8 °C on a 12:12 L:D cycle: *Porphyra nereocystis* Anderson (from Friday Harbor, Washington), *P. perforata* J. Ag. (Stanley Park, Vancouver, B.C.), and *P. variegata* (Kjell.) Hus, *P. torta* Krish., *P. fucicola* Krish., *P. abbottae* Krish. *P. schizophylla* Hollenb., and *Bangia atropurpurea* (Roth) C. Ag. (all from Vancouver Island, B.C.).

Cultures of *P. variegata, P. fucicola, P. perforata, B. atropurpurea,* and *P. schizophylla* were started from carpospores placed in petri dishes containing calcite spar and medium. Calcite substrates were harvested for fixation and resin embedding 37 days after inoculation with carpospores.

One-year-old cultures in *Macoma* shell, which had also been started from carpospores of identified macrothalli and grown under the same experimental conditions, were cast in resin and compared (Plate II).

Resin embedding was done according to Golubic *et al.* (1970) with the following modifications: Araldite (Durcupan ACM, Polysciences) was used instead of Epon resin, dehydration steps were at 15-min intervals instead of 30-min intervals, and transfer from acetone to resin was done in steps of 2:1, 1:1, and 1:2 (each 1 h), followed by two baths in pure resin, each carried out in a 50 °C oven for 2 h. No vacuum was employed. Curing of casts and their preparation for SEM was done as described in Golubic *et al.* (1970).

Micrographs were made of those regions of each preparation where the maximum differentiation of structures was visible. Thus, the bias introduced by choice of samples for analysis was roughly equivalent for all species.

Morphometric evaluation of casts (measured from sample micrographs) was conducted as follows. (1) Two measurements were taken from every filament (conchosporangial and vegetative) to represent the widest and narrowest point of each. The paired measurements were pooled for each species, and statistically evaluated separately to determine the mean widest and mean narrowest points (with standard deviations). Thus, variation in width *among* filaments in the sample populations of conchosporangial branches and vegetative filaments for each species was determined. Then the paired width measurements within each species were further evaluated by subtracting the narrower from the wider one of each pair to find the difference. The mean difference and its standard deviation were calculated separately for conchosporangial branches and vegetative filaments. The spread of the standard deviation of this measure is an indicator of the variability in diameter *within* a given filament of the species. The results are listed in Table 1. (2) The frequency of branching of conchosporangial branches and vegetative filaments was assessed by measuring the length of a given filament axis, from branch point to branch point and from branch point to filament tip. Means ($\bar{x}$) and standard deviations (SD) were calculated for each species. The number of filaments measured (n) varied according to the number of structures that were clearly visible in the sample micrographs. The results are summarized in Table 1.

Results

The endolithic conchocelis (Plate I, 1) often has both vegetative filaments (arrow) and conchosporangial branches. They are usually in the approximate size relationship illustrated. However, the actual sizes of each structure is different from species to species, with the conchosporangial branches having a wider range of sizes than the vegetative filaments (Table 1).

In this study, when one-month-old conchocelis germinated from carpospores was evaluated, the casts (Plate I, 2–5) were obviously formed by vegetative filaments. The structures germinating from *P. schizophylla* carpospores, however, developed a beaded morphology (Plate I, 6). Smooth, branching, filamentous casts were formed by this species in the year-old cultures (Plate II, 6, 8). Hence, there is some confusion as to which of the two structural types is analogous to vegetative filaments in *P.*

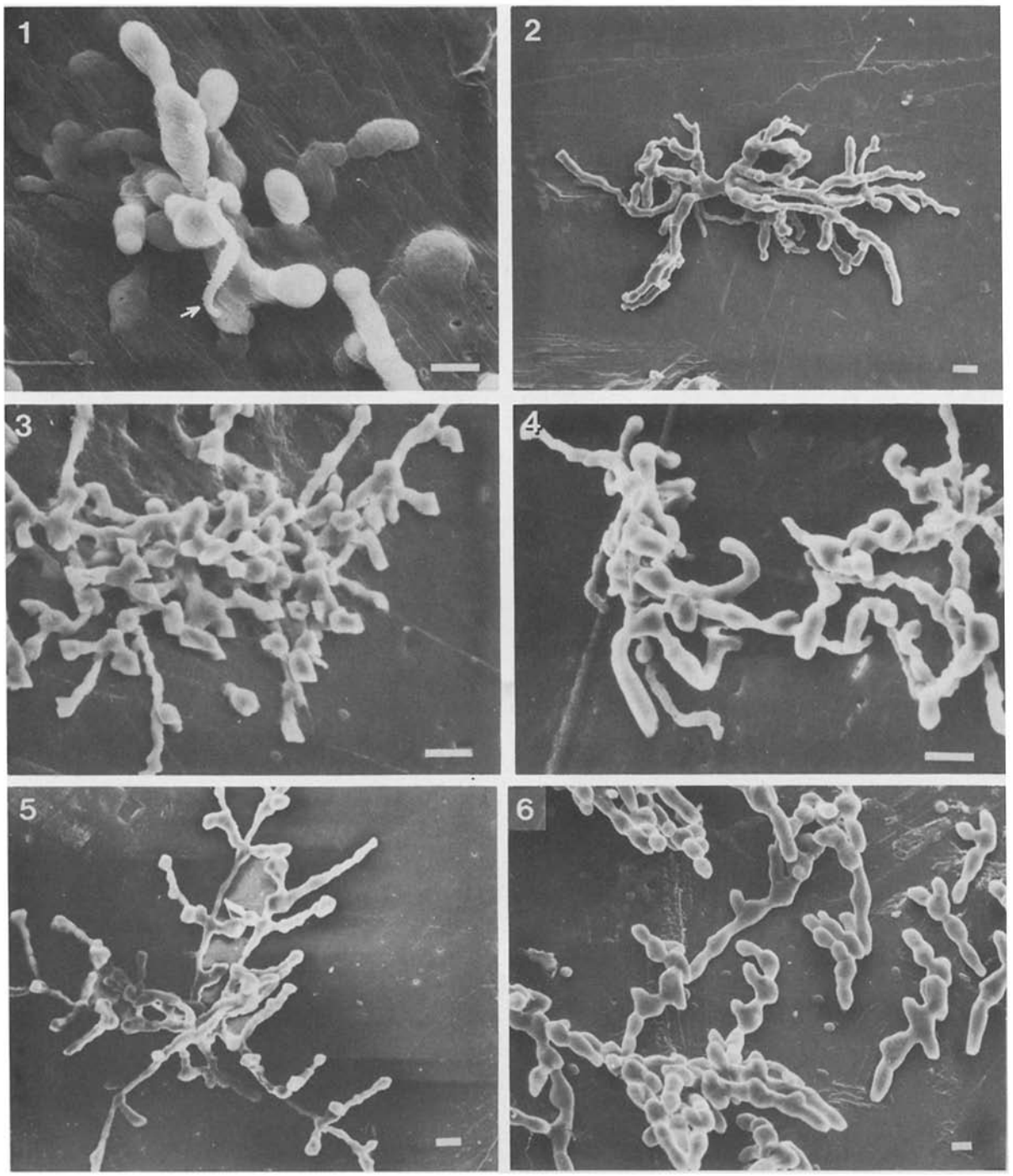

Plate I. Scanning electron micrographs of resin casts of one-year-old (Fig. 1) and one-month-old (Figs. 2–6) conchocelis borings. Scale = 10 μ. Fig. 1 shows conchocelis grown in a shell substrate, Figs. 2–6 in calcite spar (mineral has been removed from casts by HCl treatment). Fig. 1. *P. abbottae* conchosporangial branches and vegetative filament (arrow). Figs. 2–5. Vegetative filaments. Fig. 2. *P. variegata*, Fig. 3. *P. fucicola*, Fig. 4. *P. perforata*, Fig. 5. *B. atropurpurea*. Fig. 6. *P. schizophylla*, filaments of questionable function.

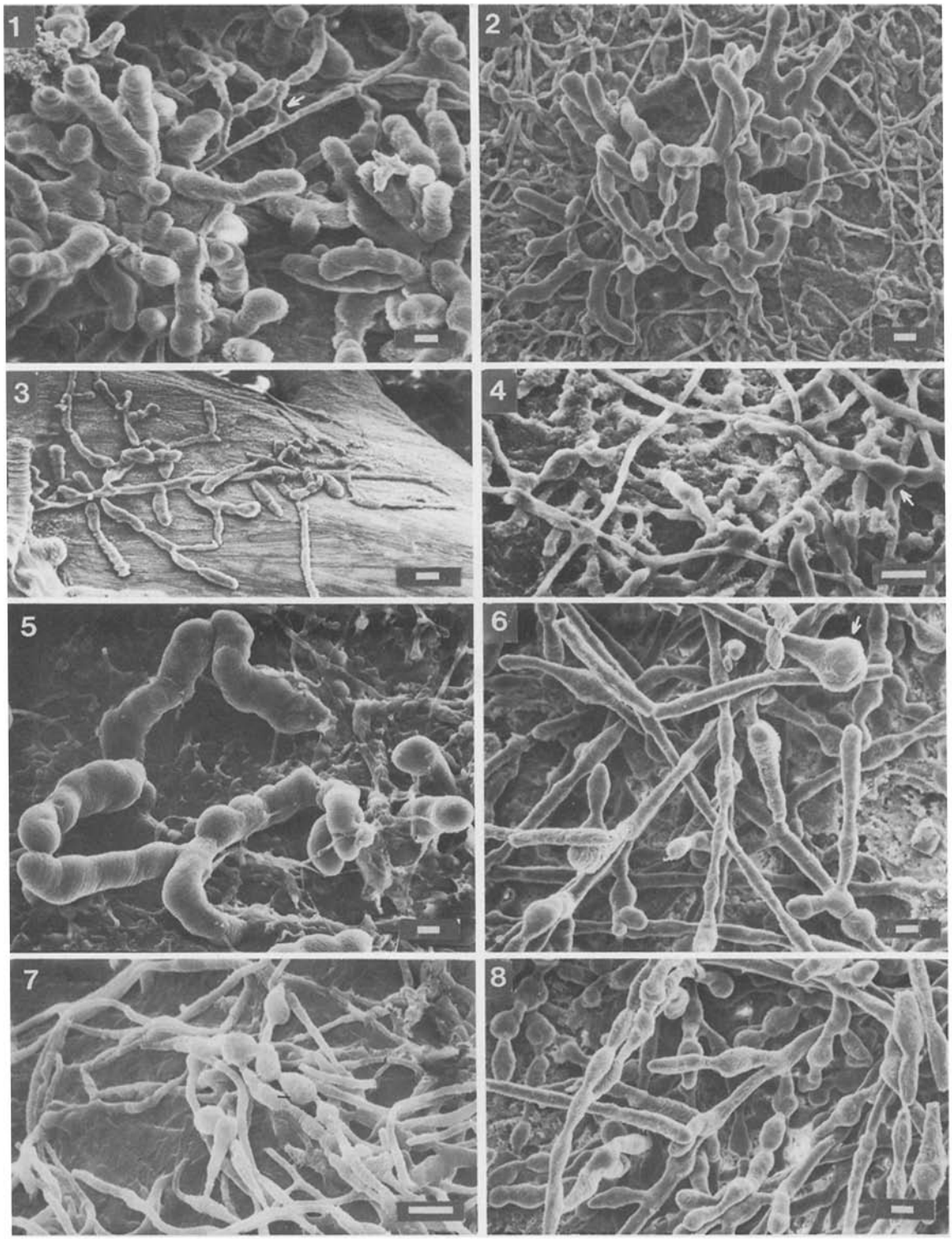

Plate II. Scanning electron micrographs of conchosporangial branch casts and vegetative filament casts of conchocelis grown in *Macoma* shell for one year. Scale bar = 10 μm. Figs. 1, 3. *P. abbottae* (arrow shows characteristic constriction of secondary branch and associated swelling on main axis); Figs. 2, 4. *P. torta* (arrow shows swelling at point of branching); Figs. 5, 7. *P. nereocystis* (note swellings along vegetative filament). Figs. 6, 8. *P. schizophylla* (arrow shows newly discovered large terminal swelling, possible conchosporangial branch equivalent). For each species, the upper figure illustrates conchosporangial branches, the lower on vegetative filaments.

schizophylla.

The one-year-old cultures of *P. abbottae, P. torta, P. nereocystis,* and *P. schizophylla* (Plate II), all showed structures attributable to vegetative filaments, conchosporangial branches, and in some cases bulbous swellings (see Campbell, 1980 for terminology).

We found that the use of calcite as an experimental substrate (see Plate I, 2–6) had an undesirable effect on the cast morphology of some species, although others showed little or no effect. *P. fucicola* (Plate I, 3) was most strongly affected, its cast morphology reflecting the faceted surface of the calcite crystallites that were etched out during boring (cf. Golubic *et al.,* 1970, Figs. 2 & 3 for similar features).

Macoma shell was found to be the most suitable of the shell substrates tested, as it contained the least amount of organic matter, and that which was present could usually be removed from casts (after acid dissolution of the shell mineral) by careful micromanipulation, or with a short chemical treatment of the casts with sodium hypochlorite (Clorox). There was no distortion of cast morphology by the shell, although the fine nacreous layering of the shell structure was sometimes reflected as a submicron-size parallel patterning over cast surfaces, as is visible on the conchosporangial branches of *P. abbottae* (Plate II, 1, 3).

Table 1 summarizes morphometric features of the resin cast boreholes of the eight species examined. The diameters of vegetative filaments are not significantly different from species to species, with the exception of *P. schizophylla* whose vegetative filaments (whether they are the beaded or the smooth branched filaments) are approximately twice as large as those of any of the other species. A distinctive monopodial, angled, opposing branching pattern was shared by *B. atropurpurea* and *P. schizophylla,* whereas *P. variegata* tended to branch in a monopodial, angled, alternating pattern. No apparent branching pattern was found for the vegetative filaments of other species. The frequency of branching was higher in vegetative filaments of the one-month-old cultures than in those one year in age. Within each of these two groups, it was found that: 1. *P. perforata* was most highly branched, and *B. atropurpurea* least; 2. *P. torta* was most highly branched and *P. nereocystis* least.

The one-year-old cultures (Plate 2) were found to have a much greater wealth of morphological and developmental information than the one-month-old cultures (Plate I, 2–6). The data have been conceptualized and presented in schematic form in Text figure 1. Additional information is included in comments, organized by species below.

P. abbottae (Plate II, 1, 3; Text fig. 1A); conchosporangial branches are more or less cylindrical, branching mainly at the base of a cluster. Very elongate axes tend to orient perpendicular to the substrate surface (geopetally?). Vegetative filaments tend to vary more in diameter than do conchosporangial branches. The secondary branches of the vegetative filament casts show constrictions at or near points of branching (arrow), whereas the primary axes tend to widen slightly at the branch point. Branching of vegetative filaments tends to be subdichotomous.

P. torta (Plate II, 2, 4; Text fig. 1B): conchosporangial branches, roughly cylindrical in shape, are the smallest in diameter of all species studied. They are about the same width as the smooth, branched filaments of *P. schizophylla* (vegetative filaments?). The main difference in the conchosporangial branch morphologies of *P. torta* and *P. abbottae* is the branching pattern. In *P. torta,* the main axes arch, with secondary branches of finite length branching in a unilateral fashion. The vegetative filaments of *P. torta* are very constant in diameter like those of *P. nereocystis,* but the swellings that develop along *P. torta* vegetative filaments at or near points of branching (arrow), are much more irregular in size and shape than those of *P. nereocystis.* Branching of vegetative filaments often occurs at right angles.

P. nereocystis (Plate II, 5, 7; Text fig. 1C): the conchosporangial branch resin casts tend to undulate in diameter, reflecting the positioning of rounded cells within them (thus, the pea-pod appearance). Their branching is dichotomous to subdichotomous. Vegetative filaments branch only rarely. Bulbous swellings develop in chains along unbranched filaments, though sometimes branching occurs at or near swellings.

P. schizophylla (Plate II, 6, 8; Text fig. 1D): it is still unclear which of the three differentiated structures is analogous to those of the other species. Large (20–24 μm diam.) club-shaped terminal swellings (arrow) are conspicuous. It is possible that these swellings represent a highly simplified type of

conchosporangial branch. However, Cole and Conway (1980) found similar large 'apical cells' to develop directly into protothalli. They also compared the beaded structure to conchosporangial branches, but they did not observe the smooth filaments with their distinctive monopodial, angled, opposing pattern. Further research will be needed to clarify these points.

Discussion

Fukuhara (1968) recognized that evaluation of the conchocelis phase can provide valuable taxonomic information. He studied cultured conchocelis to gather additional criteria to delineate and confirm the validity of previously described (macroscopically determined) taxa in Japan. Ogata (1955) made the first combined field and laboratory studies of the endolithic conchocelis of *Porphyra tenera* and *Bangia fuscopurpurea* by light microscopy of thinly ground calcite and limestone affixed to glass slides, which had been exposed to colonization and boring by conchocelis. Ogata (1961) also tested various types of mineral substrate (cement, calcite, aragonite, limestone), reporting that the conchocelis morphology developing within them was identical to that in oyster shell, the substrate traditionally employed. This result differs from ours, probably because the method of evaluation available to Ogata (low power light microscopy) did not allow the same resolution of features seen by scanning electron microscopy of resin-cast borings at higher magnification, as we used.

By 1969 the prevailing view which had developed was that the conchocelis, if found in nature, must be cultured to regenerate the macroscopic phase if it is to be identified (see Dixon & Richardson, 1969). Although some characteristic morphological features of fully differentiated conchocelis cultured from carpospores of identified macrothalli were noted by Krishnamurthy (1969) and recently by Conway & Cole (1977), it has been said that there are insufficient differences between the conchocelis of different species for reliable identification of the naturally occurring conchocelis (Kurogi 1953; Bird *et al.* 1972).

We have now demonstrated that, under standardized conditions of growth, the fully differentiated, resin-cast borehole morphologies of the cultured conchocelis of *Porphyra abbottae, P. torta, P. nereocystis,* and *P. schizophylla* are different and characterizable. We predict that when new species (and different environmental conditions) are tested, it will be possible to develop a means of taxonomic identification of many of them which will be applicable for culture as well as field work, and independent of the presence of the macroscopic phase.

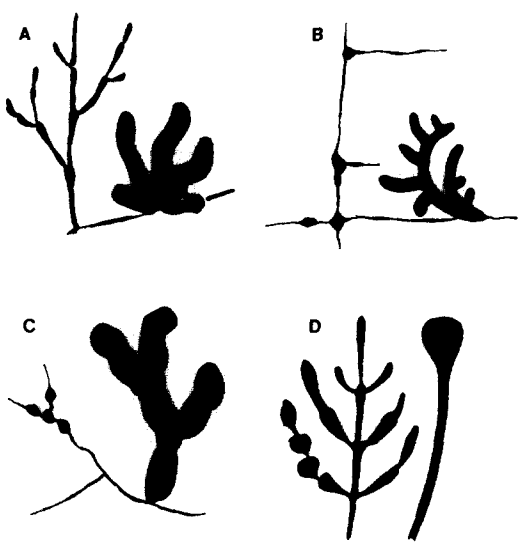

Fig. 1. Conceptualized schematic drawing of the conchocelis cast morphology of four species of *Porphyra.* Vegetative filaments (left), conchosporangial branches (right). A. *P. abbottae,* B. *P. torta,* C. *P. nereocystis,* D. *P. schizophylla.* Size relations of the drawings are approximate.

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