Botanica Marina
Vol. 31, pp. 299–305, 1988

Observations on the Marine Unicellular Endophyte *Chlorochytrium porphyrae* (Chlorophyceae)

J. A. West[1], C. M. Smith[2] and D. L. McBride[3]

Department of Botany, University of California, Berkeley, California 94720, U.S.A.

(Accepted 12 April 1988)

Abstract

Chlorochytrium porphyrae Setchell *et* Gardner is an endophytic, unicellular green alga occurring in the intertidal red alga *Porphyra perforata* of Pacific North America. *Chlorochytrium porphyrae* appears to have a haplontic *Chlamydomonas*-type of life history with biflagellate isogametes and quadriflagellate swarmers. Aplanospore-like structures are also produced *in vitro*. In culture *Chlorochytrium porphyrae* grows in other *Porphyra* species as well as artificial substrates, although it is not observed in other hosts in nature. Reproductive and cytological characteristics suggest that this alga is better placed in the genus *Halochlorococcum* (Chlorococcales) as *Halochlorococcum porphyrae* (Setchell *et* Gardner) *comb. nov.*

Introduction

Chlorochytrium porphyrae Setchell *et* Gardner was described by Setchell and Gardner in Gardner (1917) as a unicellular endophyte of *Porphyra perforata* J. Agardh. It is recorded from British Columbia to California (Scagel 1966, Abbott and Hollenberg 1976) and has not been observed in any other biogeographic region nor have aspects of its biology been investigated previously.

Unicellular endophytic green algae have been studied extensively and demonstrate widely varying life histories. Some taxonomic entities are considered 'form genera-species' or 'chlorochytrium stages'. For example, *Chlorochytrium inclusum* Kjellman and *Codiolum petrocelidis* Kuckuck, endophytes in certain foliose Rhodophyta widely distributed in the North Atlantic and North Pacific, are known to be the sporophytic phases of *Acrosiphonia* and *Spongomorpha* (Chihara 1969, Kornmann 1973, Miyaji and Kurogi 1976). By comparison, *Chlorocystis* is represented in the North Atlantic marine environment as *C. cohnii* (Wright) Reinhard and has a 'heteromorphic' unicellular life history according to Kornmann and Sahling (1983).

The type species of *Chlorochytrium* is *C. lemnae* Cohn, an endophyte in the freshwater angiosperm *Lemna*, and is clearly different from marine species of *Chlorochytrium* in that vegetative cell divisions are exhibited, whereas all the other species are unicellular (Lewin 1984). On the basis of cell division patterns and flagellar features O'Kelly and Floyd (1984) place *Chlorochytrium lemnae* in the order Chlorosarcinales.

No other taxa closely allied to *Chlorochytrium porphyrae* are recorded from the North Pacific, although other species are known from marine and freshwater environments (Kornmann and Sahling 1983, Lewin 1984). Additionally, no other *Chlorochytrium* species are known to be alternate phases in the life histories of larger, multicellular Chlorophyta. Tanner (1981) presents a more complete discussion of this matter.

The taxonomic placement of these green algae is further complicated by the genus *Halochlorococcum* Dangeard which includes five species with *H. marinum* Dangeard as the type species. Dangeard (1965) estab-

[1] To whom reprint requests should be addressed.
[2] Present address: Department of Botany, 3190 Maile Way, University of Hawaii, Honolulu, Hawaii, 96822 U.S.A.
[3] Present address: East Kootenay Community College, Cranbrook, B.C., VIC5L4, Canada

lished *Halochlorococcum* to include any unicellular, spherical, marine Chlorophyceae having a single nucleus and a reticulate or lobed chloroplast with a single pyrenoid. *Halochlorococcum moorei* (Gardner) Kornmann *et* Sahling is the only endophytic species. Some of the problems relating to the taxonomic delimitations of this genus are discussed by Guillard *et al.* (1975).

The availability of *Chlorochytrium porphyrae* in our field collections at two separate sites, as well as the lack of information on this taxon, warranted the further investigations described here.

Material and Methods

Collections of *Porphyra perforata* were made in the upper intertidal zones at Horseshoe Cove of the Bodega Marine Reserve, Sonoma County, California and Hopkins Marine Station, Monterey County, California during the years of 1984 to 1987. Blades were transported in closed plastic bags, with or without seawater-moistened paper toweling. They were stored in the same condition at 15 °C, 14:$\bar{1}$0 LD and subdued, cool white, fluourescent light (< 5 µmol m^{-2} s^{-1}) for one to seven days until examined. Small (3 – 4 mm) square blade sectors were excised and placed in sterile seawater. Released swarmers were then transferred by micropipet to 70 mm × 50 mm Pyrex[c] crystallizing dishes with 50 ml half-strength (10 ml/l) Provasoli's enriched seawater (McLachlan 1973) and cultured at 15 °C, 14:$\bar{1}$0 LD, 10 – 15 µmol mm^{-2} s^{-1}. In addition to examination of plants growing on the inner surface of the culture vessel, cover slips were floated on the water surface and were removed periodically for microscopic observation. Similar cultures were initiated at 10 °C and 20 °C.

For studies of host specificity, field collected blades of *Porphyra perforata, P. lanceolata* (Setchell *et* Hus) Smith, *P. nereocystis* Anderson and *P. schizophylla* Hollenberg were cut as 1 cm discs and placed in similar culture systems as described above (15 °C, 14:$\bar{1}$0 LD). Experimental discs were inoculated with cells from the *Chlorochytrium* cultures and periodic observations were made over the next several weeks. Conchocelis stages of *P. perforata* and *P. nereocystis* in unialgal culture were also inoculated with *Chlorochytrium* cell suspensions. In addition, *Chlorochytrium* cell suspensions were streaked on 1% agar made with half-strength Provasoli's medium. Observations were made on a Zeiss GFL bright field microscope with Neofluar lenses and attached phase contrast system. Light micrographs were exposed with a Zeiss Jena automatic exposure photomicrographic apparatus with an 80B filter using Kodak Panatomic X film.

Numerous unsuccessful attempts were made to obtain a reliable chemical fixation of *Chlorochytrium* for transmission electron microscopy.

Observations and Discussion

Porphyra perforata specimens collected in the mid- to upper-intertidal region above mussel beds at the Bodega and Hopkins sites (Fig. 1) showed a light green color, whereas thalli about 0.5 m lower were the more normal purplish-red color. During microscopic observations, it was apparent that the vegetative cells of the *Porphyra* blades infected with *Chlorochytrium* were often a distinct yellow-green color, characteristic of cells with reduced phycobiliprotein levels. Indeed, some *Porphyra* thalli appeared completely bleached and dead, while *Chlorochytrium* was still viable in these host tissues. In all cases, it was evident that the endophyte preferred host plants which were smaller and somewhat tattered (Fig. 2), a condition perhaps reflecting environmental stress. *Audouinella porphyrae* (Drew) Garbary is a filamentous endophyte of *Porphyra perforata* and frequently occurred with *Chlorochytrium* in *Porphyra* tissues.

Chlorochytrium cells were either crowded or uniformly spaced in infected areas of the blades and, either partially or totally, embedded in the cell wall beneath the cuticle (Figs 3, 4). None were observed between the host cells as with *Chlorochytrium inclusum* in other foliose red algae (Chihara 1969) or *Codiolum petrocelidis* in *Petrocelis* (Hollenberg 1958, Fan 1959). Various developmental stages were usually evident in field collected *Porphyra perforata* (Figs 5, 6, 7). The cells

Figs 1 – 7.
Fig. 1. Collection site for *Chlorochytrium porphyrae* at Hopkins Marine Station (Monterey County, California). The infected *Porphyra perforata* plants are toward the top of the rocky outcropping, while normal, uninfected plants appear on the sides. Fig. 2. An infected *Porphyra* plant appears at the top of this figure compared with an uninfected plant below. Scale bar = 2 cm. Figs. 3 and 4. Cross sections of an infected *Porphyra* thallus illustrating embedded *Chlorochytrium* cells (arrows) and their relationship to host cells. Scale bar = 20 µm (for Figs 3 – 8). Fig. 5. Surface view of infected thallus illustrating closely packed, nonreproductive *Chlorochytrium* cells. The single lobed, peripheral chloroplast appears axial in surface view because of its orientation within the cell. Fig. 6. Two celled stage during swarmer production. Fig. 7. Additional stages in swarmer production. The maximum number of swarmers per reproductive cell seemed to vary due to undivided cells being present during development (arrow).


1
2
3
4
5
6
7

ranged from the smallest vegetative cells (5 μm diameter) to full sized reproductive cells (25 to 40 μm diameter). Each cell contained a single peripheral, lobed chloroplast with a conspicuous pyrenoid projecting into the vacuole. The chloroplast is oriented toward maximum light (i. e. away from the host plant thallus), thus only in surface view the chloroplast appears axial and stellate (Fig. 5). The contents of the large reproductive stages were subdivided into at least 64 small cells. Flagellated cells were released from these large reproductive structures immediately after immersion of the partially dried blades into seawater (Fig. 8). Under optimal conditions (20 °C, 16:$\bar{8}$ LD, 20 μmol cm^{-2} s^{-1}), 12 to 16 days were required for a cell to undergo eleutheroschisis and reach reproductive maturity. Occasionally one or more cells within the parent cell failed to divide which resulted in fewer than the maximum number of cells (Fig. 7). It was not possible to synchronize the cell division sequence in culture even though several combinations of temperature, photoperiod, light intensity and nutrient level changes were tested.

The swarmers released from the host thallus escaped singly (not in an enclosed vesicle or gelatinous mass) through a conspicuous, rimmed discharge pore (10 to 15 μm diameter) (Fig. 9). Occassionally an attached operculum was observed after swarmer release. Rarely, however, were these structures persistent (Fig. 10). Biflagellate swarmers were obovoid to pyriform, 4 to 6 μm long and 2 to 3 μm wide (Fig. 11), and exhibited anterior fusion (Fig. 12). The quadriflagellate swarmers tended to be more spherical (Fig. 13) then rounded up after settling. Each swarmer contained a single cup-shaped chloroplast with a lateral or posterior pyrenoid and an antero-lateral eyespot. The flagellated cells did not appear to exhibit any strong phototactic response. Both types of flagellated cells were released from field and cultured materials, although biflagellate forms appeared more frequently, particularly in field specimens. Many generations of *Chlorochytrium* have been followed in culture, thus the above observations seem to suggest that this plant has the classic haplontic *Chlamydomonas*-type of life history.

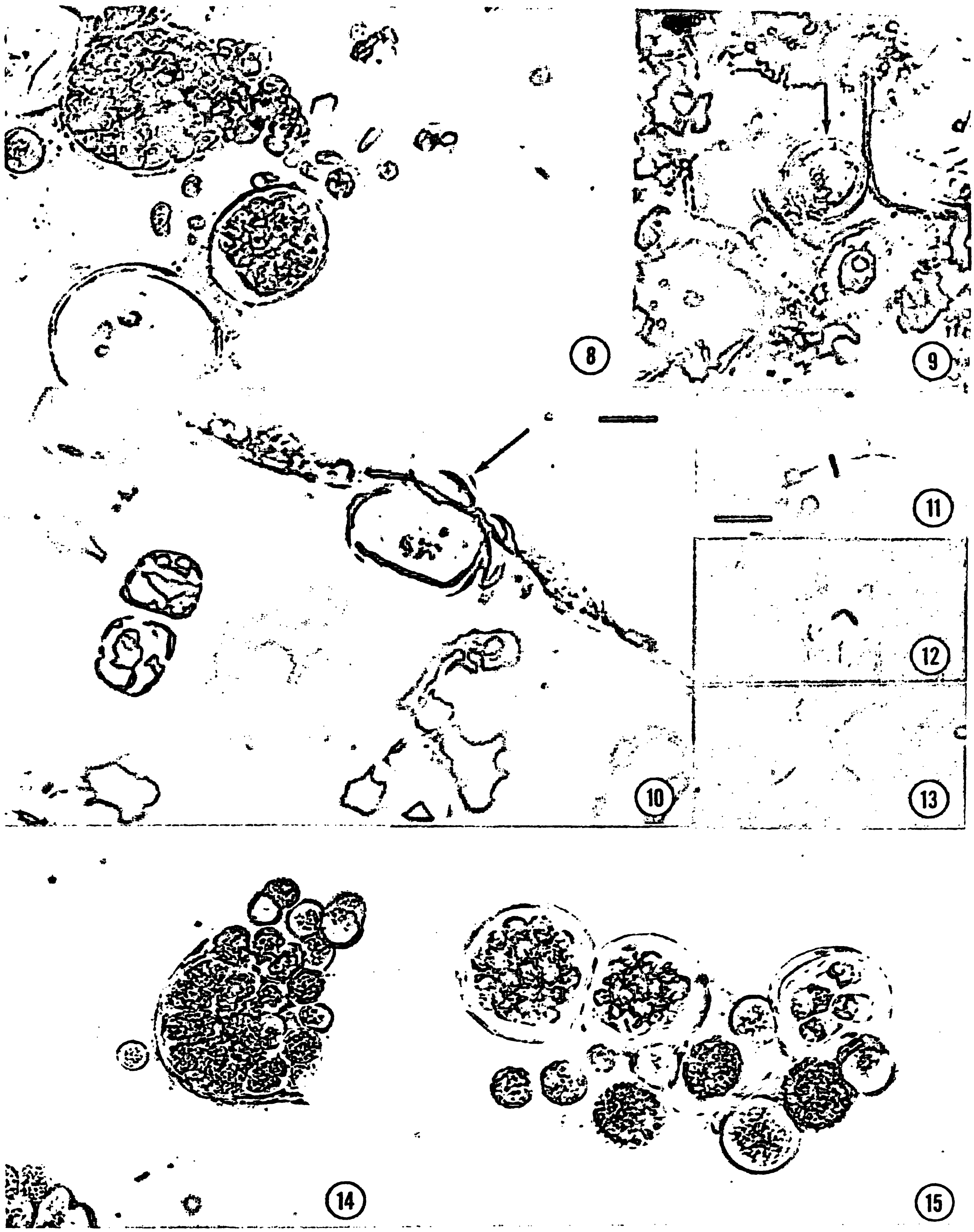
Under the stress of nutrient depletion, older cultures of *Chlorochytrium* formed cyst-like stages with notably thicker cell walls (Fig. 14). This process occurred not only in single vegetative cells, but also in cells undergoing eleutheroschisis. This phenomenon appears similar to that attributed to *Chlorococcum* (Bold and Wynne 1985). The resultant walled 'aplanospores' are released when the original 'parent cell' wall is broken (Fig. 15).

When cells were grown on agar media, the division sequence was initiated when the cell diameters were smaller than the diameters of cells grown in liquid media. For example, an 8-celled stage could be only 16 μm in diameter. On agar the reproductive stage formed non-motile aplanospores that usually germinated *in situ*. *Chlorochytrium* grew slowly on agar media compared with host-free liquid cultures which demonstrated relatively rapid growth on glass and plastic surfaces.

In each of the four species of *Porphyra* (*P. perforata*, *P. lanceolata*, *P. schizophylla*, and *P. nereocystis*) tested for host specificity, *Chlorochytrium* was able to occupy the outer cell wall matrix but did not penetrate between the cells. For 12 days of host specificity observations, the growth of the discs punched from three of the *Porphyra* thalli (*P. perforata*, *P. lanceolata* and *P. schizophylla*) not inoculated with *Chlorochytrium* showed that intertidal species were not damaged by disc punching treatments. In contrast, discs from *P. nereocystis* showed damage, especially in freshly cut margins. In addition, *Chlorochytrium* was unable to attach to or penetrate the cell walls of the conchocelis phases of either *P. perforata* or *P. nereocystis*. Presumably, the differences in the physical and chemical structure of the cell wall between the conchocelis and the blade stages of *Porphyra* (Gretz *et al.* 1982, 1983) may allow the conchocellis phase to resist the *Chlorochytrium* infection.

The above culture results suggest that host specificity is not exclusive for *Chlorochytrium porphyrae* although it appears to be restricted to one host in nature. Other *Porphyra* species have been examined, both in the field and the herbarium and no *Chlorochytrium* has been found infecting these plants. In view of reports by other authors (see Kornmann and Sahling 1983), *Blidingia* and *Enteromorpha* also would appear to be acceptable hosts for *Halochlorococcum moorei*, which occurs naturally in these green algae and can be cultured in the laboratory on *Blidingia minima* (Kützing) Kylin. However, *Chlorochytrium porphyrae* did not attach to the Kornmann isolates of *Blidingia minima* in culture (Kornmann and Sahling 1983).

In the original description of *C. porphyrae* (Gardner 1917), mature vegetative and reproductive cells were described as 40 to 60 μm in diameter and the biflagellate gametes fusiform to spherical, 3 to 4 μm in diameter and escaping through an oval opening in the outer wall. The swarmer dimensions are smaller than we have observed in either field or cultured material. Gardner made no mention of the discharge pore or of the occurence of quadriflagellate swarmers.



Figs 8–15.
Fig. 8. Release of swarmers. Fig. 9. Surface view of rimmed discharge pore (arrow). Fig. 10. Embedded reproductive cell after spore release. Note the operculum associated with the discharge pore. Scale bar = 15 µm (for Figs 9 and 10). Fig. 11. Pyriform biflagellate swarmer (phase contrast view). Scale bar = 10 µm (for Figs 11–13). Fig. 12. Fusion of isogametes (phase contrast). Fig. 13. Quadriflagellate swarmer (phase contrast). Fig. 14. Thick walled cells grown in nutrient deficient culture media. Fig. 15. Release of aplanospore-like cells from thick- walled 'parent cell' grown in nutrient deficient culture media.

Table I. Comparisons of features for *Chlorochytrium porphyrae* and *Halochlorococcum moorei.*

Host specificity	H. moorei	C. porphyrae
Field	*B. minima*[1]	*P. perforata* [3,4,5]
Laboratory	N/A	*P. perforata*[4,5] *P. lanceolata*[4,5] *P. nereocystis*[4,5] *P. schizophylla*[4,5]
Vegetative cells		
chloroplast	peripheral and lobed[1,2]	peripheral and lobed[4]
pyrenoid	1/cell[1]	1/cell[4]
Cytokinesis	possible 64 cells[1]	successive 2X multiples, 64 or more cells[4]
Motile cells		
types	swarmers[1]	isogametes and swarmers[4]
no. flagella	four[1]	two and four[4]
eyespots	one lateral anterior[1,2]	one lateral anterior[4]
discharge site	oval opening (12 to 20 μm)[6] with operculum without rim[1,2]	oval opening (10 to 15 μm) with rim and operculum[4]
phototaxis	negative[2]	neutral[4]

[1]Kornmann and Sahling (1983)
[2]observations on *Halochlorococcum* cultures
[3]Setchell and Gardner (1917)
[4]observations on *Chlorochytrium* field collections and cultures (this paper)
[5]gametophyte phases only
[6]measurements taken from figures in Kornmann and Sahling (1983)

These structures are observed in *Halochlorococcum* (Kornmann and Sahling 1983). In addition, many other features are shared by *Chlorochytrium* and *Halochlorococcum* (Table I).

Conclusions

Halochlorococcum moorei as described by Kornmann and Sahling (1983) and earlier investigators (Dangeard 1965, Guillard *et al.* 1975), fits the general size, vegetative and reproductive details of *Chlorochytrium porphyrae*. The primary differences are that *H. moorei* occurs on *Blidingia*, that the swarmers show strong, negative phototaxis (Kornmann personal communication) (a characteristic not exhibited by those of *C. porphyrae*) and that spore sizes differ. As a result of our investigations, it seems likely that host affiliations are not exclusive and may be of questionable taxonomic importance. To resolve this taxonomic problem definitively, comparative studies of the life histories of *Halochlorococcum* and *Chlorochytrium* as well as host specificity investigations are needed using culture isolates from Atlantic and Pacific populations (the major geographic distribution of these algae). Other areas of investigation could certainly include comparative cell and flagellar base ultrastructure, also protein and cell wall polysaccharide immunology. Perhaps cryo-preservation techniques would be of use for transmission electron microcopy.

At this point it is appropriate that a taxonomic decision be made and it is our view that *Chlorochytrium porphyrae* Setchell *et* Gardner should be known as *Halochlorococcum porphyrae* (Setchell *et* Gardner) *comb. nov.* Placement in a specific order must await fine structural evidence on the flagellar apparatus and cytokinesis.

Formal transfer
Halochlorococcum porphyrae (Setchell *et* Gardner) West comb. nov.
Basionym: *Chlorochytrium porphyrae* Setchell *et* Gardner in Gardner 1917, p. 379, Pl. 32, Fig. 6.

Acknowledgements

Financial support for this investigation was provided to the first author by the Committee on Research, University of California, Berkeley. The second author thanks the Miller Foundation for Basic Research in Science, University of California, Berkeley, for a two year research fellowship. The third author acknowledges financial support from East Kootenay Community College, Cranbrook. We also gratefully acknowledge Dr Peter Kornmann for cultures of *Halochlorococcum moorei* and *Blidingia minima* and valuable comments on the distinctions between *H. moorei* and *Chlorochytrium porphyrae*.

References

Abbott, I. A. and G. J. Hollenberg. 1976. *Marine Algae of California*. Stanford University Press. 827 pp.

Bold, H. C. and M. J. Wynne. 1985. *Introduction to the Algae.* 2nd. Ed. Prentice Hall, Inc., N.J. 720 pp.

Chihara, M. 1969. Culture study of *Chlorochytrium inclusum* from the northeast Pacific. *Phycologia 8*: 127–133.

Dangeard, P. 1965. Sur deux Chlorococcales marines. *Le Botaniste 58*: 65–74.

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.

Gardner, N. L. 1917. New Pacific coast marine algae. I. *Univ. Calif. Publ. Bot. 6*: 377–416.

Gretz, M., E. McCandless, J. Aronson and M. Sommerfeld. 1983. The galactan sulphates of the conchocelis phases of *Porphyra leucosticta* and *Bangia atropurpurea* (Rhodophyta). *J. Exp. Bot. 34*: 705–711.

Gretz, M., M. Sommerfeld and J. Aronson. 1982. Cell wall composition of the generic phase of *Bangia atropurpurea* (Rhodophyta). *Bot. Mar. 25*: 529–535.

Guillard, R., H. Bold and F. MacEntee. 1975. Four new unicellular chlorophycean algae from mixohaline habitats. *Phycologia 14*: 13–25.

Hollenberg, G. J. 1958. Observations concerning the life cycle of *Spongomorpha coalita* (Ruprecht) Collins. *Madroño 14*: 249–251.

Kornmann, P. 1973. Codiolophyceae, a new class of Chlorophyta. *Helgolander wiss. Meeresunters. 25*: 1–13.

Kornmann, P. and P.-H. Sahling. 1983. Meeresalgen von Helgoland: Ergänzung. *Helgolander Meeresunters. 36*: 1–65.

Lewin, R. 1984. Culture and taxonomic status of *Chlorochytrium lemnae*, a green algal endophyte. *Br. phycol. J. 19*: 107–116.

McLachlan, J. 1973. Growth media-marine. *In*: (J. R. Stein, ed.) *Handbook of Phycological Methods* Culture Methods and Growth Measurements. Cambridge Univ. Press. England.

Miyaji, K. and M. Kurogi. 1976. On the development of zoospores of the green alga *Chlorochytrium inclusum* from eastern Hokkaido. *Bull. Jap. Soc. Phycol. 24*: 121–129.

O'Kelly, C. J. and G. L. Floyd. 1984. Flagellar apparatus absolute orientations and the phylogeny of the green algae. *Biosystems 16*: 227–251.

Scagel, R. 1966. *Marine Algae of British Columbia and Northern Washington. Part I. Chlorophyceae (Green Algae)*. Bull. No. 207. Nat. Mus. Canada. 257 pp.

Setchell, W. and N. Gardner. 1920. The marine algae of the Pacific coast of North America. Part III. Chlorophyceae. *Univ. Calif. Publ. Bot. 8*: 139–374.

Tanner, C. 1981. Chlorophyta: life histories. *In*: (C. Lobban and M. Wynne, eds) *Biology of Seaweeds*. Univ. of Calif. Press. Los Angeles. 218–247 pp.