

## Distribution of green light-harvesting pigments, siphonaxanthin and siphonein, and their precursors in marine green algae

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The distribution of green light-harvesting pigments, siphonaxanthin and siphonein, and their precursors, lutein and loroxanthin, was investigated in about 40 species of marine benthic green algae collected from various habitats. Among the members of the Ulvales, Cladophorales and Siphonocladales a large amount of lutein was detected only in shallow-water species, whereas a large amount of loroxanthin or siphonaxanthin was in deep-water species. In the eusiphonean orders, Codiales, Derbesiales and Caulerpales, siphonaxanthin and siphonein were detected in all species, and a small amount of lutein or loroxanthin was present in some species collected from shallow waters. In *Dichotomosiphon tuberosus*, the only species studied belonging to the Dichotomosiphonales, collected from sunny site in fresh-water, considerable amounts of lutein, loroxanthin and siphonein were detected. The results implies that the eusiphonean orders originated in deep waters. The fact that some species of the Caulerpales were found on sand at depths exceeding 30 m suggests that the caulerpalean algae common on sand in shallow lagoon have come from sand in deep waters.

The pigment composition of the eusiphonean algae, as well as the life style of the caulerpalean algae on sand, may be in the most ancestral condition among green algae since the Chlorophyta is concluded to have appeared about one thousand million years ago a time when strong ultraviolet rays at a lethal level penetrated 5 to 10 m into seawater.

*Key Index Words:* Caulerpales—Cladophorales—Codiales—Derbesiales—Dichotomosiphonales—loroxanthin—lutein—siphonaxanthin—siphonein—Siphonocladales—Ulvales.

Siphonaxanthin and its ester, siphonein, have been found in a number of siphonous green algae (Strain 1951, 1965, Jeffrey 1965, Kleinig and Egger 1967, Kleinig 1969). It was reported by Kleinig (1969) that both siphonaxanthin and siphonein were present in all species examined of the three eusiphonean orders, Codiales, Derbesiales and Caulerpales, whereas in a fourth order Dichotomosiphonales, only siphonein was present, and in some species of the orders Cladophorales and Siphonocladales, only

siphonaxanthin was present. He regarded this specific pigment distribution as an aid in the classification of the siphonous green algae.

However, Yokohama and his colleagues have reported that siphonaxanthin is characteristic of most deep-water green algae, and that siphonaxanthin and siphonein function as photosynthetic pigments harvesting green light which prevails in deep waters (Kageyama *et al.* 1977, Yokohama *et al.* 1977, Kageyama and Yokohama 1978). The function of siphonaxanthin was further confirmed by Anderson (1983) who isolated a light-harvesting siphonaxanthin-chlorophyll *a/b*-protein complex from a *Codium* species.

Yokohama (1981a) examined the distribution of siphonaxanthin and siphonein in about 50 species of marine green algae with special reference to their habitats. It was confirmed that in all species of the eusiphonean orders examined, siphonaxanthin and/or siphonein was present. In the other orders, the distribution of siphonaxanthin was restricted within species found in deep or shaded sites.

As pointed out by Yokohama (1981a, 1981b, 1982), the fact that all the members of the eusiphonean orders have siphonaxanthin and/or siphonein implies that they originated in deep waters, and in fact, many species of the eusiphonean orders were collected by trawling from the depths of 50 m or more (Ueda and Okada 1938). Although many species of these orders inhabit sunny sites in shallow waters, the amount of siphonaxanthin and its ester, contained in them, is so small that these pigments seem to be relics. It is very interesting that most members of the Caulerpales have well developed fibrous rhizoids which anchors them on unstable sand. Many species of this order can be found in calm lagoons surrounded by reefs. Their original habitat may be a sandy place in deep calm waters.

The present authors have searched for species of Caulerpales inhabiting sand in deep waters. In the spring of 1988, six species of Caulerpales and one of Codiales were found on sand at depths over 30 m in the southernmost part of Japan. In the present paper, the distribution of siphonaxanthin, siphonein and their precursors, lutein and loroxanthin, in marine green algae, including those algae collected from sand in deep waters, is reported.

### Materials and Methods

Most species of Caulerpales and Dichotomosiphonales and some species of other orders were collected at the Yaeyama Islands, in the southern part of Japan. Seven species (*Codium* sp., *Caulerpa filicoides*, *C. lentilifera*, *C. subserrata*, *Udotea javensis*, *Tydemania expeditionis* and *Halimeda opuntia* f. *opuntia*)

were collected from sand at depths of 34 or 37 m in Amitori Bay, Iriomote Island in Yaeyama Islands. Some of these are shown in Fig. 1.

Most species of orders other than Caulerpales and Dichotomosiphonales were collected at Shimoda in Central Japan. Collected thalli were soaked in a large volume of seawater and transported to the Shimoda Marine Research Center, where pigment extraction was undertaken. Species collected at the Yaeyama Islands were transported frozen.

Both fresh and frozen material was extracted with cold methanol which was subsequently mixed with a nearly equal volume of diethylether. Pigments were transferred to the ether layer by shaking with a 10% sodium chloride solution. After repeated washing, with 10% sodium chloride, the ether layer was evaporated under reduced pressure and residue was redissolved in a small volume of ether.

Chromatography of the pigments was carried out on thin-layer plates of Kieselgel 60 (Merck). A mixture of petroleum ether 30°-60° and acetone (7 : 3, v/v) was used as the developing solvent. Each of xanthophylls and chlorophyll *a* was eluted by a solvent from thin-layer chromatogram under a stream of nitrogen. The solvent for xanthophylls was ethanol and that for chlorophyll *a* was diethylether.

Absorbances of pigments were measured at their absorption maxima by a Shimadzu UV-3000 spectrophotometer with eluates from the thin-layer chromatograms for each species, and their quantities were calculated using the following molar absorption coefficients: 91.0 for chlorophyll *a*, 145.0 for lutein, 103.0 for loroxanthin and 69.7 for siphonaxanthin. For the absorption coefficient for siphonein, the value of siphonaxanthin was tentatively used as that of siphonein is unknown. Although the molar ratio of each xanthophyll to chlorophyll *a* was calculated using these values, that of chlorophyll *b* to chlorophyll *a* was calculated from absorbances of the pigment mixture in ether, at 642.5 nm and 660 nm, using the equation of Comar and

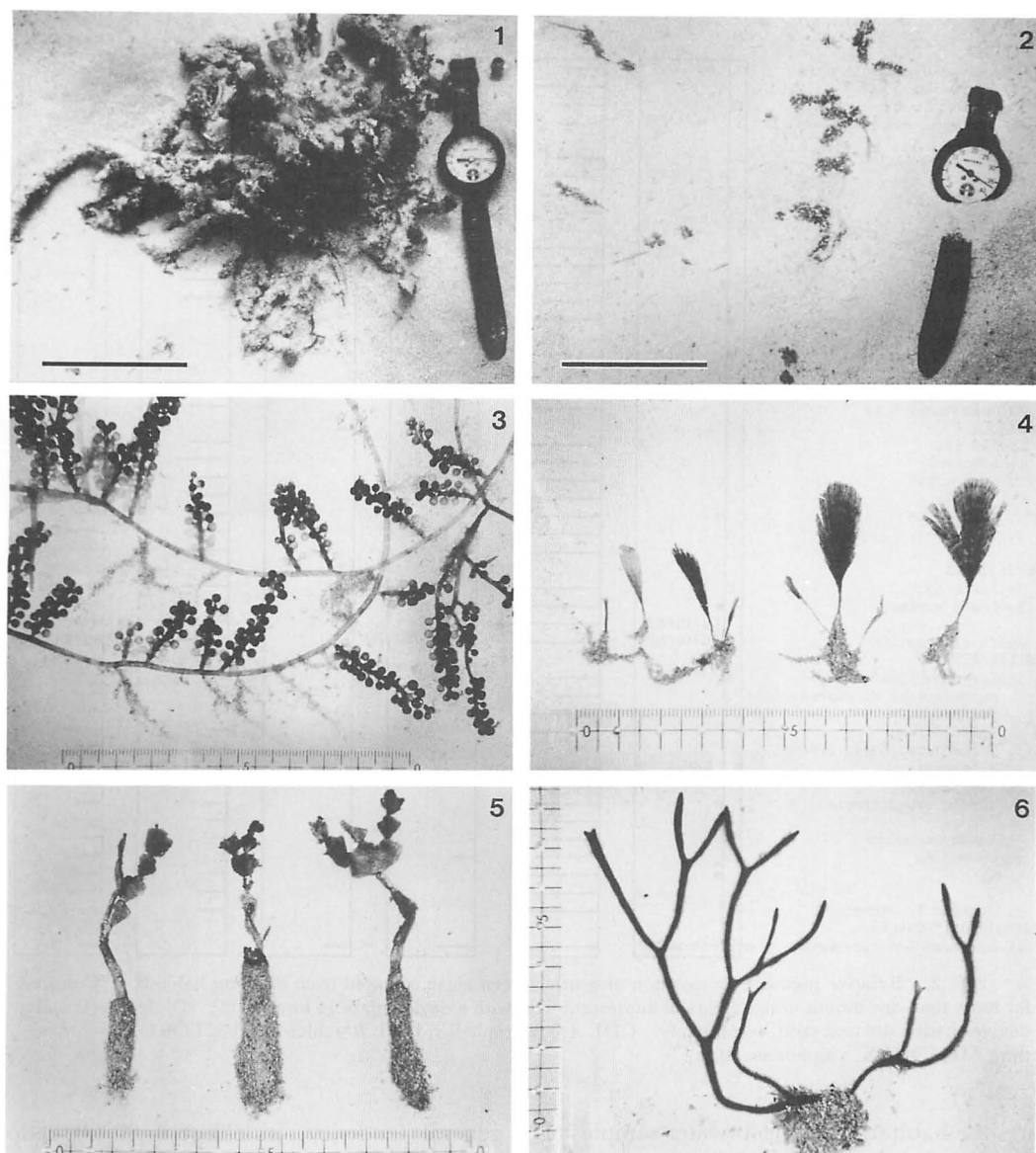


Fig. 1. Green algae of the eusiphonean orders from sand at depths over 30 m off Amitori of Iriomote Island in Okinawa Prefecture. 1, *Tydemania expeditionis* on sand at 34 m; 2, *Caulerpa lentillifera* on sand at 37 m; 3-6, *C. lentillifera*, *Udotea javensis*, *Halimeda opuntia* and *Codium* sp. respectively collected from sand at 37 m. Bar in 1, 2=15 cm; minimum division of scale in 3-6=1 mm.

Zsheile (1942) and their molecular weights.

## Results

Fig. 2 shows the molar ratios of the xanthophylls to chlorophyll *a*, determined in about 40 species of green algae collected from

different habitats. The ratio of chlorophyll *b* to chlorophyll *a* is also shown, since it is closely correlated with the light field of the habitat (Yokohama 1973, Yokohama *et al.* 1977, Kageyama and Yokohama 1978, Yokohama and Misonou 1980).

As can be seen in the column which indi-

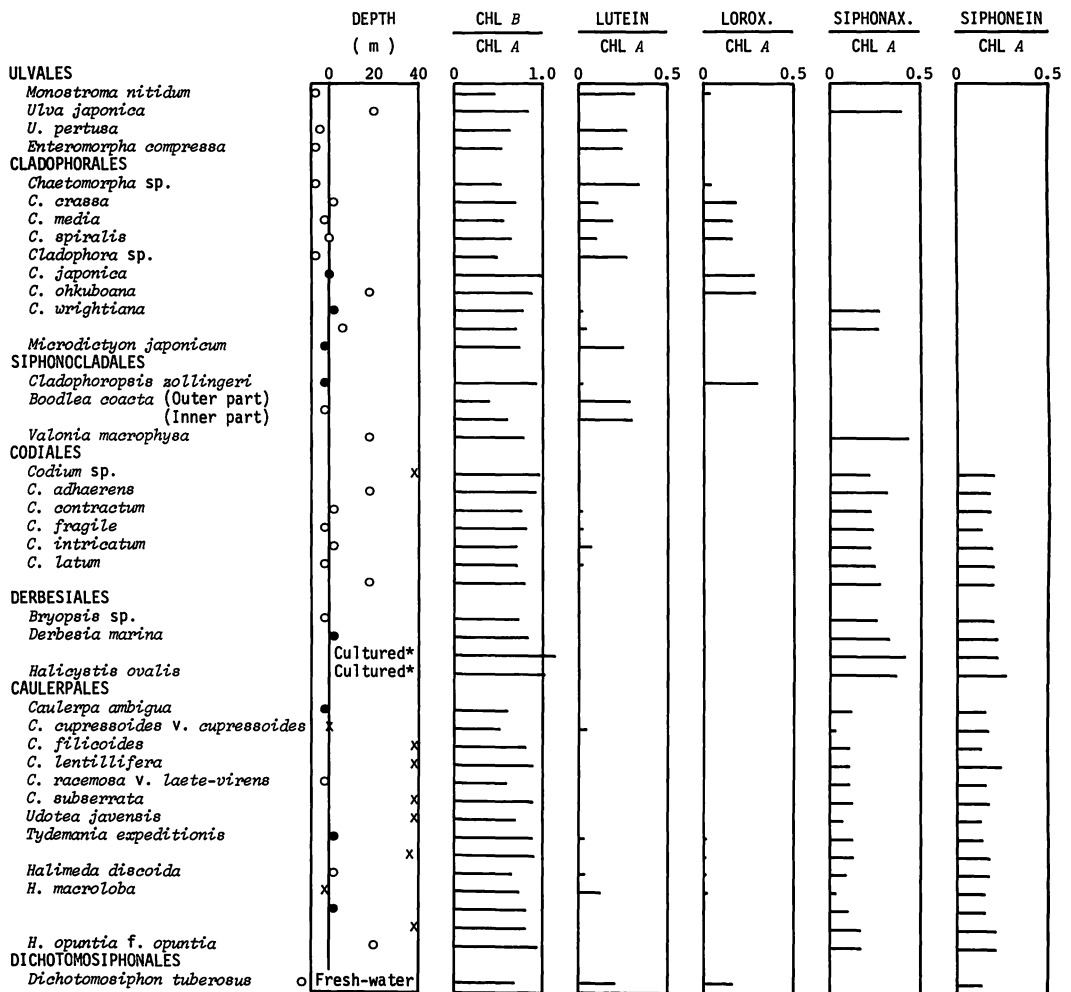


Fig. 2. Relative pigment composition of benthic green algae collected from different habitats. \*Cultured for more than one month under 2 klux of fluorescent light with a daylength of 14 hr at 20 °C. Closed circle and x denote shaded site and sand, respectively. CHL A = chlorophyll a; CHL B = chlorophyll b; LOROX. = loroxanthin; SIPHONAX. = siphonaxanthin.

cates the habitat, some deep-water samples of the eusiphoncean orders grow on sand. Among these *Codium* sp. is especially remarkable. It was collected from sand at a depth of 37 m and had delicate fibrous rhizoids as shown in Fig. 1-6. All other species of *Codium*, including deep-water ones, have sucker-shaped rhizoids and grow on rocks. *Tydemania expeditionis* is also an interesting species. It was collected from sand at 34 m depth, as shown in Fig. 1-1, while in shallow water it occurs on rocks or dead coral.

The second column of Fig. 2 indicates a

general tendency, in chlorophyll *b/a* ratio, that samples collected from deeper or shaded sites have higher components of chlorophyll *b*. The other columns indicate that the distribution of xanthophylls is clearly correlated with habitat in the upper three orders, the Ulvales, Cladophorales and Siphonocladales. In these orders, the green light-harvesting pigment, siphonaxanthin, is found in algae inhabiting deep or shaded sites, while lutein, the precursor of siphonaxanthin, is found in algae inhabiting sunny sites in shallow water, although a trace amount of lutein was

Table 1. Distribution of xanthophylls in the seven orders of green algae.

Order	Habitat	Lutein	Loroxanthin	Siphonaxanthin	Siphonein
Ulvales	Sun	+	Trace or -	-	-
Cladophorales	Deep or Shade	Trace or -	+		
Siphonocladales				-	+
Codiales	Sun	Trace or -	Trace or -	+	+
Derbesiales	Deep or Shade	-	-		
Caulerpales					
Dichotomosiphonales	Sun	+	+	-	+

detected in a deep-water species, *Cladophora wrightiana*, which contained a large amount of siphonaxanthin.

From these results it is clear that deep-water species contain a large amount of siphonaxanthin and shallow-water species contain a large amount of lutein. However, there are some deep-water species lacking siphonaxanthin and containing a large amount of loroxanthin, the intermediate product from lutein to siphonaxanthin. These also lacked lutein. There are some shallow-water species containing both lutein and loroxanthin. The total amount of lutein and loroxanthin in these species seems equivalent to that of lutein, loroxanthin or siphonaxanthin in the other species. The molar ratios of these pigments to chlorophyll *a* are around 0.3.

*Microdictyon japonicum* collected from a shaded site is the exceptional species that contained a large amount of lutein regardless of habitat. However, it had a ratio of chlorophyll *b* to chlorophyll *a* equivalent to other species collected from deep or shaded sites.

Although in the upper three orders (Fig. 2), siphonaxanthin is restricted to algae inhabiting deep water or shaded shallow sites, the distribution of this pigment and its ester, siphonein, is apparently not correlated with habitat in the lower four orders. Both pigments are present in all members of Codiales, Derbesiales and Caulerpales regardless of habitat, and siphonein is present in *Dichotomosiphon tuberosus* collected from a sunny site in fresh-water. Lutein and loro-

xanthin are present in some members of these orders inhabiting sunny sites in shallow waters. However, the amounts of these pigments are very small in species other than *Dichotomosiphon tuberosus*.

Green algae cited in this study can be divided into three groups as shown in Table 1, according to the correlation between pigment composition and habitat. The first consists of the Ulvales, Cladophorales and Siphonocladales; the second and third are of the eusiphonous orders, the Codiales, Derbesiales, Caulerpales (second group) and Dichotomosiphonales (third group). *Dichotomosiphon tuberosus*, which is the only species studied belonging to the Dichotomosiphonales, is unique among the eusiphonous orders in inhabiting fresh-water and having a large amount of lutein and loroxanthin, while lacking siphonaxanthin.

## Discussion

It is clearly shown that the distribution of the green light-harvesting pigment, siphonaxanthin, has an ecological significance in the Ulvales, Cladophorales and Siphonocladales, as indicated in the upper half of Fig. 2, while in the eusiphonous orders, the distribution of this pigment and another green light-harvesting pigment, siphonein, seems taxonomically significant. The fact that all members of the eusiphonous orders have the green light-harvesting pigment(s) implies that these groups of green algae originated in deep waters. In fact, seven species of these orders are found

on sand at depths exceeding 30 m. Six belong to the Caulerpales. This suggests that the caulerpalean algae in shallow water, most of which are common on sand in lagoons, have come from sand in deep waters.

A surprising fact is that a member of the Codiales, *Codium* sp., inhabits sand at a depth of 37 m. It has fibrous rhizoids which enable it to grow on sand, while all other species of Codiales, including deep-water species, have sucker-shaped rhizoids and inhabit rocks. The occurrence of *Codium* sp. on sand in the deep water might indicate the primitive life style of the Codiales.

In the Cladophorales and Siphonocladales there are unique algae lacking siphonaxanthin, despite of their inhabiting deep or shaded sites. They have a large amount of loroxanthin, the direct precursor or siphonaxanthin. It is remarkable that lutein, the precursor of loroxanthin, is nearly or completely absent in deep-water species while is abundant in shallow-water species having neither loroxanthin nor siphonaxanthin. O'Kelly (1982), who analyzed the chloroplast pigments of the marine chaetophoracean and chaetosiphonacean algae, noticed that species having siphonaxanthin, but lacking lutein, are restricted to deeper water habitats. Lutein seems essential to some shallow-water green algae, as pointed out by Yokohama (1981b, 1982). It was suggested by Yokohama (1983) that this xanthophyll might protect the photosynthetic apparatus from short wavelength or ultraviolet light prevailing in shallow sunny sites.

Yokohama (1981b, 1982, 1983) suggested that deep-water species having loroxanthin instead of siphonaxanthin might have originated from deep-water species with siphonaxanthin, by failing to oxidize loroxanthin into siphonaxanthin. In the same fashion, shallow-water species with lutein might have originated from deep-water species with loroxanthin or siphonaxanthin.

It is reasonable to regard the ancestor of green algae as a deep-water alga possessing green light-harvesting pigment(s): Barghoorn and Schopf (1965) concluded that the Chlo-

rophyta appeared about one thousand million years ago, a time when strong ultraviolet rays at a lethal level probably penetrated 5 to 10 m into seawater (Barkner and Marshall 1965).

From the systematic viewpoint, it is also accepted that the ancestral green alga might have possessed siphonaxanthin. Ricketts (1970, 1971) reported that this pigment is widely distributed in scaly green monads, regarded by Stewart and Mattox (1978) as the nearest extant relatives of the ancestral green alga. Therefore, O'Kelly (1982) regarded the presence of siphonaxanthin as the ancestral condition, and Anderson (1983) regarded this xanthophyll as an evolutionary relic in some Chlorophyta.

The pigment composition of eusiphonacean algae, as well as the life style of caulerpalean algae on sand, may be in the most ancestral condition among green algae.

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#### References

- Anderson, J. M. 1983. Chlorophyll-protein complexes of a *Codium* species, including a light-harvesting siphonaxanthin-chlorophyll *a/b*-protein complex, an evolutionary relic of some Chlorophyta. *Biochim. Biophys. Acta* **724**: 370-380.
- Barghoorn, E. S. and Schopf, J. W. 1965. Microorganisms from the late Precambrian of Central Australia. *Science*, N.Y. **150**: 337-339.
- Barkner, L. V. and Marshall, L. C. 1965. On the origin and rise of oxygen concentration in the earth's atmosphere. *J. Atmos. Sci.* **22**: 225-261.
- Comar, C. L. and Zscheile, F. P. 1942. Analysis of plant extracts for chlorophylls *a* and *b* by a photoelectric spectrophotometric method. *Plant Physiol.* **17**: 198-201.
- Jeffrey, S. W. 1965. Pigment composition of Siphonales algae in the brain coral *Favia*. *Biol. Bull.* **135**: 141-148.
- Kageyama, A. and Yokohama, Y. 1978. The function of siphonin in a siphonous green alga *Dichotomisiphon tuberosus*. *Jap. J. Phycol.* **26**: 151-155.
- Kageyama, A., Yokohama, Y., Shimura, S. and Ikawa, T. 1977. An efficient excitation energy transfer from a carotenoid, siphonaxanthin to chlorophyll *a*

- observed in a deep-water species of chlorophycean seaweed. *Plant & Cell Physiol.* **18**: 477-480.
- Kleinig, H. 1969. Carotenoids of siphonous green algae: A chemotaxonomical study. *J. Phycol.* **5**: 281-284.
- Kleinig, H. and Egger, K. 1967. Zur Struktur von Siphonaxanthin und Siphonein, den Hauptcarotinoiden siphonaler Grünalgen. *Phytochem.* **6**: 1681-1686.
- O'Kelly, C. J. 1982. Chloroplast pigments in selected marine Chaetophoraceae and Chaetosiphonaceae (Chlorophyta): The occurrence and significance of siphonaxanthin. *Bot. Mar.* **25**: 133-137.
- Ricketts, T. R. 1970. The pigments of the Prasinophyceae and related organisms. *Phytochemistry.* **9**: 1835-1842.
- Ricketts, T. R. 1971. Identification of xanthophylls KI and KIS of the Prasinophyceae as siphonein and siphonaxanthin. *Phytochemistry.* **10**: 161-164.
- Stewart, K. D. and Mattox, K. R. 1978. Structural evolution in the flagellated cells of green algae and land plants. *Biosystems* **10**: 145-152.
- Strain, H. H. 1951. The pigment of algae. p. 243-262. *In* G. M. Smith [ed.] *Manual of Phycology*. Chronica Botanica Co., Waltham, Mass.
- Strain, H. H. 1965. Chloroplast pigments and the classification of some siphonalean green algae of Australia. *Biol. Bull.* **129**: 366-370.
- Ueda, S. and Okada, Y. 1938. Studies on the vegetation of the marine algae in the seas of Japan, with special reference to the depth of the growing-zone. *Bull. Jap. Sci. Fish.* **7**: 229-236.
- Yokohama, Y. 1973. Photosynthetic properties of marine benthic green algae from different depths in the coastal area. *Bull. Jap. Soc. Phycol.* **21**: 70-75.
- Yokohama, Y. 1981a. Distribution of the green light-absorbing pigments siphonaxanthin and siphonein in marine green algae. *Bot. Mar.* **24**: 637-640.
- Yokohama, Y. 1981b. Green light-absorbing pigments in marine green algae, their ecological and systematic significance. *Jap. J. Phycol.* **29**: 209-222.
- Yokohama, Y. 1982. Distribution of lutein and its derivatives in marine green algae. *Jap. J. Phycol.* **30**: 311-317.
- Yokohama, Y. 1983. A xanthophyll characteristic of deep-water green algae lacking siphonaxanthin. *Bot. Mar.* **26**: 45-48.
- Yokohama, Y. and Misonou, T. 1980. Chlorophyll *a*: *b* ratios in marine benthic green algae. *Jap. J. Phycol.* **28**: 219-223.
- Yokohama, Y., Kageyama, A., Ikawa, T. and Shimura, S. 1977. A carotenoid characteristic of chlorophycean seaweeds living in deep coastal waters. *Bot. Mar.* **20**: 433-436.

横浜康継\*・平田 徹\*・御園生 拓\*\*・田中次郎\*\*\*・横地洋之\*\*\*\*：海産緑藻における  
緑色光捕獲色素 siphonaxanthin および siphonein とそれらの前駆物質の分布

種々の環境から得た約40種の海産底生緑藻について緑色光捕獲色素とそれらの前駆物質の分布を調べた結果、緑色光捕獲色素がクダモ類中の調べた種すべてに含有されていることが明らかとなった。この事実はクダモ類すなわちミル目・ツクノイト目・イワヅタ目・チョウチンミドロ目が深所起源であることを示唆している。クダモ類は浅所にもかなり分布し、とくにイワヅタ目中の多くの種は浅いが波静かな礁湖底の砂上という特異な環境に生育しているが、同一種あるいは近縁種が深度30mを超す砂質の海底に見出されることから、これらの藻群の本来の生育地は深所の砂地と考えられる。緑藻は約10億年前に出現したとされていることから、始源緑藻は強い紫外線の到達しない深所に生育していたと考えられるが、深所に卓越する緑色光を捕獲する色素を含有し静かな砂上に定着しているクダモ類は、始源緑藻に最も近い特徴を具えているものとみなせよう。(\*415 下田市5-10-1 筑波大学下田臨海実験センター, \*\*400 甲府市武田 山梨大学教育学部生物学教室, \*\*\*160 東京都新宿区百人町2-23-1 国立科学博物館植物研究部, \*\*\*\*907-15 沖縄県八重山郡竹富町崎山 東海大学海洋研究所西表分室)

