Jap. J. Phycol. (Sôrui) 28: 75-79. June 20, 1980

Plastid pigments of *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus[1)]

Yasutsugu YOKOHAMA*, Takaaki KOBARA**
and Mitsuo CHIHARA**

* *Shimoda Marine Research Center, The University of Tsukuba, Shimoda, Shizuoka-ken, 415 Japan*
** *Institute of Biological Sciences, The University of Tsukuba, Ibaraki, 305 Japan*

YOKOHAMA, Y., KOBARA, T. and CHIHARA, M. 1980. Plastid pigments of *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus. Jap. J. Phycol. 28: 75-79.

The pigment composition of *Pseudodichotomosiphon constrictus* was compared with those of *Dichotomosiphon tuberosus* belonging to the Chlorophyceae and *Vaucheria vipera* belonging to the Xanthophyceae. The result obtained shows the similarity of the pigment composition between *P. constrictus* and *V. vipera*, both contain at least chlorophylls *a* and *c*, carotene(s) and diadinoxanthin. There was no detection in these algae of either fucoxanthin characteristic of the Phaeophyceae, Crysophyceae and Bacillariophyceae or chlorophyll *b* characteristic of the Chlorophyceae, Prasinophyceae and Euglenophyceae. These evidences suggest affinity of *P. constrictus* with the Xanthophyceae.

Key Index Words: *Chlorophyll* a; *chlorophyll* c; *diadinoxanthin*; *plastid pigments*; Pseudodichotomosiphon; *systematic position*; *Xanthophyceae*.

It has been shown in our previous paper (HORI *et al.* 1979) with the aid of electron microscope that *Pseudodichotomosiphon constrictus* (YAMADA) YAMADA possesses ultrastructural features fundamentally identical with those of the Chromophyta. For obtaining further information of this alga, the plastid pigments have been examined. For comparison, the pigments of *Dichotomosiphon tuberosus* ERNST and *Vaucheria vipera* BLUM have also been analyzed. The present paper gives a result of our investigation of the composition of the plastid pigments, together with some remarks regarding the systematic position of the genus *Pseudodichotomosiphon*.

Materials and Methods

The localities and dates of the collection of specimens used in the present study are shown in Table 1. The specimens were maintained in Provasoli's enriched seawater (prepared according to MCLACHLAN 1973) at 20°C, 14-10 h photoperiod, using cool-white fluorescent illumination (c. 2500-3000 lx).

The specimens were ground with cold methanol in a glass homogenizer. Homogenates were filtered through a glassfiber filter, and the extraction was repeated several times until the residue became colorless. The combined methanol extract was mixed with a nearly equal volume of diethylether in a separatory funnel. The pig-

1) Contributions from the Shimoda Marine Research Center, No. 361.

Table 1. Organisms used in the investigation

Organisms	Collecting localities	Dates
Dichotomosiphon tuberosus	Oyama, Okinawa (Freshwater)	Apr. 27, 1978
Pseudodichotomosiphon constrictus	Minamihama, Okinawa (Middle intertidal zone)	Apr. 26, 1978
Vaucheria vipera	Nashiro, Okinawa (Lower intertidal zone)	Apr. 25, 1978

ments were transferred to the ether layer by shaking with a 10% NaCl solution. After repeating wash with NaCl solution, the ether layer was dried up under reduced pressure, and the residue was redissolved in a small volume of ether.

The pigments were separated by cellulose thin-layer chromatography. A mixture of n-hexane and methyl ethyl ketone (4 : 1 v/v) was used as the developing solvent.

Pigments to be examined spectrophotometrically were eluted from the chromatograms in n-hexane, diethylether or ethanol. Absorption spectra were determined with a Shimazu UV-200 Spectrophotometer.

Results

The thin-layer chromatograms of pigments of *D. tuberosus*, *P. constrictus* and *V. vipera* are shown in Fig. 1. From this figure, we can recognize significant difference in pigment composition between *D. tuberosus* and *P. constrictus*, while the pigment composition of the latter alga is quite similar to that of *V. vipera*. As is seen in the figure, chlorophylls *a* and *b* in addition to the principal carotenoids known from siphonous green algae (KLEINIG 1969), such as carotenes, lutein, violaxanthin, siphonein and neoxanthin, are present in the pigments from *D. tuberosus*. In contrast to it, xanthophylls present in the pigments from *P. constrictus* and *V. vipera* are quite different from those in the pigments from *D. tuberosus*. *P. constructus* and *V. vipera* lack chlorophyll *b* and contain chlorophyll *c* in its place.

Four fractions of xanthophylls X. 1 to X. 4 are recognizable on the chromatogram

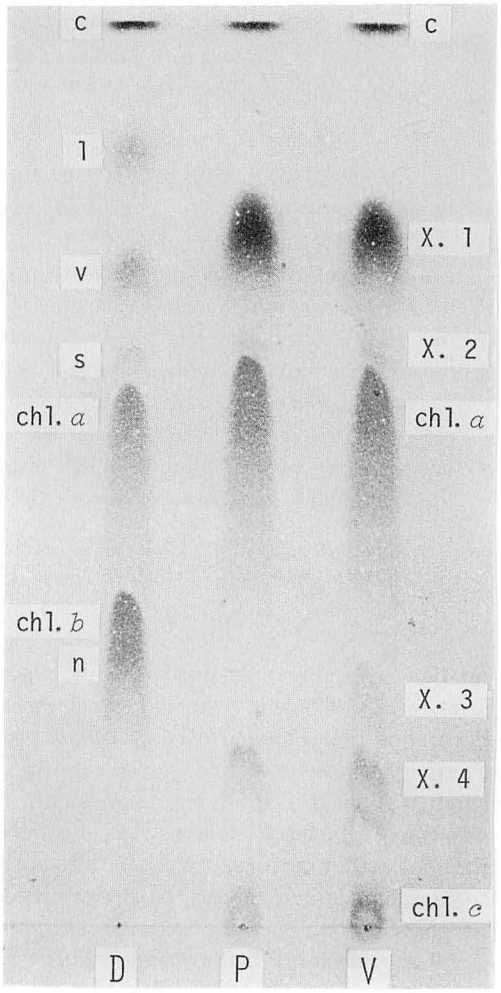

Fig. 1. Cellulose thin-layer chromatograms of pigments from *Dichotomosiphon tuberosus*, *Pseudodichotomosiphon constrictus* and *Vaucheria vipera*. The developing solvent: n-hexane and methyl ethyl ketone (4 : 1, v/v). D=*D. tuberosus*; P=*P. constrictus*; V=*V. vipera*; c=carotene (s); l=lutein; v=violaxanthin; s=siphonein; chl. *a*=chlorophyll *a*; chl. *b*=chlorophyll *b*; chl. *c*=chlorophyll *c*; n=neoxanthin; X. 1, X. 2, X. 3 and X. 4=xanthophylls from *P. constrictus* or *V. vipera*.

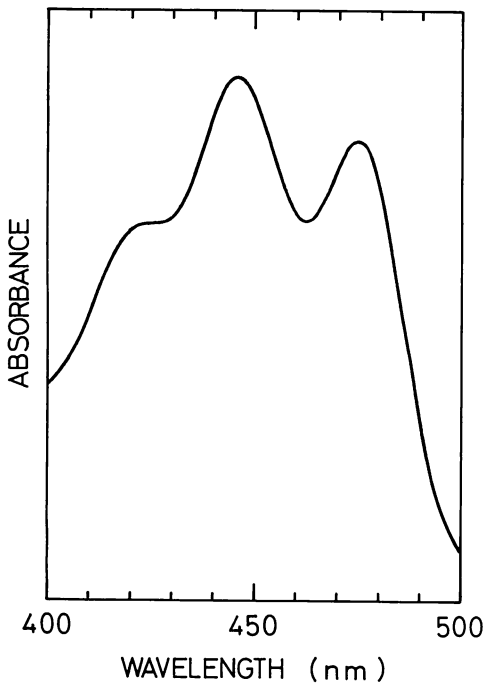

Fig. 2. The absorption spectrum of Fraction X. 1 from *Pseudodichotomosiphon constrictus* and *Vaucheria vipera*.

of *V. vipera*. Three of them X. 1, X. 2 and X. 4 are common to *V. vipera* and *P. constrictus*. Fraction X. 1 is least strongly adsorbed on the cellulose plate of those xanthophylls and estimable to constitute the majority of the total carotenoids. The absorption maxima of this fraction in ethanol locate at about 445 and 474 nm as shown in Fig. 2.

THOMAS & GOODWIN (1965) and KLEINIG & EGGER (1967) reported that antheraxanthin constituted 50% or more of the total carotenoid content both in *Tribonema* and *Vaucheria*. As diadinoxanthin was confused with antheraxanthin in those early investigations (GOODWIN 1974), diadinoxanthin can be the most abundant carotenoid. WHITTLE & CASSELTON (1975) and WHITTLE (1976) also reported that diadinoxanthin was the xanthophyll most abundant in some members of Xanthophyceae, such as *Pleurochloris meiringenis*, *Mischococcus sphaerocephalus*, *Tribonema aequale* and *Ophiocytium majus*. This pigment is least strongly adsorbed in columns or thin-layer plates of major xanthophylls produced by xanthophycean algae (STRAIN *et al.* 1968, EGGER *et al.* 1969, cf. THOMAS & GOODWIN 1965).

Fraction X. 1 is suspected to be diadinoxanthin since it can be regarded as the most abundant and the least strongly adsorbed one of the xanthophylls detected in *V. vipera*. Furthermore, this suspicion is supported by the fact that the absorption maxima of this fraction are close to those of diadinoxanthin ever reported (MANDELLI 1968, STRAIN *et al.* 1968, EGGER *et al.* 1969, STRAIN *et al.* 1970, STRANSKY & HAGER 1970). Thus we have identified X. 1 as diadinoxanthin.

The other xanthophylls of *P. constrictus* or *V. vipera* could not be identified since quantities of them eluted from the thin-layer plates were too little to determine their absorption spectra.

The fraction denoted as chl. *c* in the chromatograms of *P. constrictus* and *V. vipera* was identified to be chlorophyll *c* by determining its absorption spectrum. It was well coincident with that of chlorophyll *c* from *Tribonema aequale* reported by GUILLARD & LORENZEN (1972).

Discussion

Result obtained in the present study shows the similarity of the pigment composition between *Pseudodichotomosiphon constrictus* and *Vaucheria vipera*, both contain at least chlorophylls *a* and *c*, carotene (s) and diadinoxanthin. There was no detection in these algae of pigment fractions corresponding to either fucoxanthin or chlorophyll *b*, the former being xanthophyll characteristic of the Phaeophyceae, Chrysophyceae and Bacillariophyceae, and the latter being chlorophyll characteristic of the Chlorophyceae, Prasinophyceae and Euglenophyceae. These evidences suggest affinities of *P. constrictus* with the Xanthophyceae. On the contrary, our result has revealed the presence of chlorophylls *a* and *b*, lutein, violaxanthin, siphonein and neoxanthin in *Dichotomosiphon tuberosus*. This

clearly indicates affinities of the genus *Dichotomosiphon* with Codiales (sensu lato) of the Chlorophyceae. THOMAS & GOODWIN (1965) and KLEINIG & EGGER (1967) examined pigment compositions of certain members of the Xanthophyceae, including *Vaucheria*, and reported antheraxanthin to be in greatest abundance of all detected carotenoids, but the pigment called as "antheraxanthin" by these authors is now known as diadinoxanthin. In fact, WHITTLE & CASSELTON (1975) reported for certain xanthophycean algae that the most abundant carotenoid was diadinoxanthin. In the present study, the diadinoxanthin was also detected in large quantity in both *Pseudodichotomosiphon* and *Vaucheria*. On the basis of morphological similarity in addition to the similarity of pigment composition revealed in this study, it should be more natural to place the genus *Pseudodichotomosiphon* in the Xanthophyceae, classifying it next to the genus *Vaucheria*.

Acknowledgment

We wish to express our thanks to Mr. Shintoku KAMURA, The University of Ryukyus, for his generous aid in collecting the materials. Thanks are also due to Dr. John A. WEST, University of California, Berkeley, for his kind reading of the manuscript.

References

EGGER, K., NITSCHE, H. und KLEINIG, H. 1969. Diatoxanthin und Diadinoxanthin-Bestandteil des Xanthophyllgemisches von *Vaucheria* und *Botrydium*. Phytochemistry **8**: 1583–1585.

GOODWIN, T. W. 1974. Carotenoids and biliproteins. p. 176–205. *In* W. D. STEWART [ed.] Algal physiology and biochemistry. Blackwell Sci. Publ. Oxford, London.

GUILLARD, R. R. L. and LORENZEN, C. J. 1972. Yellow-green algae with chlorophyllide *c*. J. Phycol. **8**: 10–14.

HORI, T., KOBARA, T. and CHIHARA, M. 1979. Electron microscope observations on *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus. Jap. J. Phycol. **27**: 183–191.

KLEINIG, H. 1969. Carotenoids of siphonous green algae: A chemotaxonomical study. J. Phycol. **5**: 281–284.

KLEINIG, H. und EGGER, K. 1967. Carotinoide der *Vaucheriales Vaucheria* und *Botrydium* (Xanthophyceae). Z. Naturforschg. **22** b: 868–872.

MANDELLI, E. F. 1968. Carotenoid pigments of dinoflagellate *Glenodinium foliaceum* strain. J. Phycol. **4**: 347–348.

MCLACHLAN, J. 1973. Growth media-marine. p. 25–51. *In* J. STEIN [ed.] Handbook of phycological methods. Culture methods and growth measurements. Cambridge Univ. Press.

STRAIN, H. H., BENTON, F. L., GRANDOLFO, M. C., AITZETMÜLLER, K., SVEC, W. A. and KATZ, J. J. 1970. Heteroxanthin, diatoxanthin and diadinoxanthin from *Tribonema aequale*. Phytochemistry **9**: 2561–2565.

STRAIN, H. H., SVEC, W. A., AITZETMÜLLER, K., GRANDOLFO, M. and KATZ, J. J. 1968. Molecular weights and empirical formulas of the xanthophylls of *Vaucheria*. Phytochemistry **7**: 1417–1418.

STRANSKY, H. and HAGER, A. 1970. Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. II. Xanthophyceae. Arch. Microbiol. **71**: 164–190.

THOMAS, D. M. and GOODWIN, T. W. 1965. Nature and distribution of carotenoids in the Xanthophyta (Heterokontae). J. Phycol. **1**: 118–121.

WHITTLE, S. J. and CASSELTON, P. J. 1975. The chloroplast pigments of the algal classes Eustigmatophyceae and Xanthophyceae. II. Xanthophyceae. Br. phycol. J. **10**: 192–204.

WITTLE, S. J. 1976. The major chloroplast pigments of *Chlorobotrys regularis* (WEST) BOHLIN (Eustigmatophyceae) and *Ophiocytium majus* NÄGELI (Xanthophyceae). Br. phycol. J. **11**: 111–114.

横浜康継*・高原隆明**・千原光雄**: **クビレミドロの色素組成と分類学上の位置**

クビレミドロの色素組成をチョウチンミドロ (緑藻綱) およびウミフシナシミドロ (黄緑藻綱) の色素組成と比較したところ，クビレミドロとウミフシナシミドロとの間に共通性がみられた。両者はクロロフィル*a*およびカロチンの他に少くともクロロフィル*c*およびディアディノキサンチンを含むことが分かった。また褐藻綱・黄藻綱・珪藻綱に特有なフコキサンチンおよび緑藻綱・プラシノ藻綱・ミドリムシ綱に特有なクロロフィル*b*はウミフシナシミドロと同様にクビレミドロでも検出されなかった。これらの結果からクビレミドロは黄緑藻綱に属せしめるべきものと考えられる。(*415 静岡県下田市 5-10-1 筑波大学下田臨海実験センター・**305 茨城県新治郡桜村　筑波大学生物科学系)