

Electron microscope observations on *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus¹⁾

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The chloroplasts in the multinucleate, vegetative cell of *Pseudodichotomosiphon constrictus* has the following three ultrastructural characteristics which are thought to be useful for elucidating the systematic position of this alga; 1) three-thylakoid bands, 2) no reduction of thylakoid number per band in the pyrenoid matrix, 3) presence of superimposed layer of chloroplast endoplasmic reticulum. On the basis of these characters in addition to its gross morphology, *P. constrictus* is considered as a member of the Xanthophyceae.

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Pseudodichotomosiphon, a monotypic genus of siphonous algae, with the type species, *P. constrictus* (called as *P. constricta*), was established by YAMADA (1934) on the basis of specimens collected in Okinawa. This species was originally described by the same author (1932) as a member of the genus *Vaucheria*. Main characters used by YAMADA (1934) for separating it from *Vaucheria* are: 1) the presence of constrictions in places of the frond, 2) the possession of a rhizoidal part at the base, and 3) dichotomous ramification. However, the systematic position of the genus has been problematical. In agreement with YAMADA (1934) many taxonomists have placed the genus in the Codiales (sensu lato) of the Chlorophyceae (OKAMURA 1936, TSENG 1936, FRITSCH 1935, FOTT 1971, HIROSE 1972), whereas others had doubts about YAMADA's treatment and placed it in the Vaucheriaceae of the Xanthophyceae (LUTHER 1953, SEGAWA 1956, SEGAWA and KAMURA 1960,

YAMAGISHI 1964). Nevertheless, no one has obtained the critical information needed to determine the systematic affinity of this genus.

The present paper constitutes the first of a series of the studies on *Pseudodichotomosiphon constrictus* and presents the result of our investigation on vegetative cell ultrastructure, together with some remarks regarding its systematic position.

Materials and Methods

Specimens used in the present study were originally collected by one of us (TK) at Minami-hama, Okinawa, in March, 1978, (Fig. 1) and were maintained in PROVASOLI's enriched seawater (prepared according to McLACHLAN 1973) at 20°C, 14-10 h photoperiod, using cool-white fluorescent illumination (ca. 2500-3000 lux).

Vegetative axes were fixed at room temperature for 4 hours in 4% glutaraldehyde

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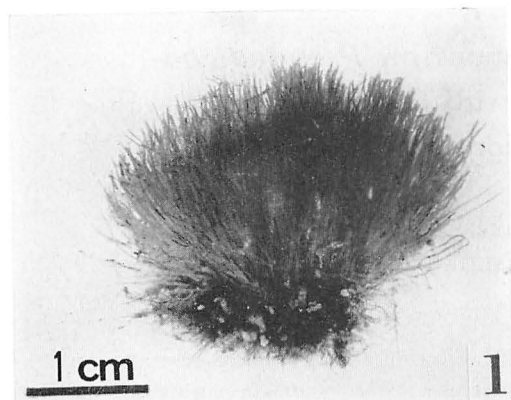


Fig. 1. *Pseudodichotomosiphon constrictus* from Okinawa.

in 0.1 M phosphate buffer at pH 7.0 containing 0.27 M sucrose. Following fixation in glutaraldehyde, the sucrose was gradually decreased by one-third step reductions in successive changes of the buffer (at least 30 minutes for each step). Postfixation was carried out using 2% osmium tetroxide in the same buffer for 5 hours. Details for dehydration processes and embedding procedure in the resin was previously described (HORI and ENOMOTO 1978).

Thin sections were made by LKB ultratome III, using glass knives and stained with REYNOLDS' lead citrate for 12 minutes (REYNOLDS 1963), and viewed in an Hitachi HU-12A electron microscope.

Results

Longitudinal section of the siphonous, multinucleate vegetative thallus shows the cell organization in *P. constrictus* (Fig. 2). The nuclei, scattered throughout the cell, are ovoid or circular in profile, measuring 1.5–2.5 μm in diameter, with a nucleolus composed of granular aggregates (Fig. 8). Chloroplasts are either rod-shaped or elliptical in profile both in thin longitudinal section (Fig. 2) and the light microscope (Fig. 3). In cross section, generally the chloroplasts look like the spatula (Figs. 4, 10). The enlarged pyrenoid portion contains a finely granular matrix which is in low density and has 7–8 lamellar bands each

consisting of three thylakoids (Figs. 2, 4, 5, 10). This end of the chloroplast is usually directed toward the central vacuole (Fig. 4). The distance between the chloroplast envelope and the outermost layer of a thylakoid band in the pyrenoid region is up to 50 nm, whereas that between two adjacent bands ranges between 200 to 300 nm. These bands converge at the proximal end of the pyrenoid matrix immediately beneath the chloroplast envelope; the tips of the thylakoids meet there and adhere tightly with one another, but do not attach directly to the chloroplast envelope (Fig. 5, arrow). Two or three bands terminate within the pyrenoid matrix (Figs. 5, 10). In some cases two bands cohere for a short distance in the pyrenoid matrix. The thylakoid bands in the pyrenoid region are continuous with those of chloroplast proper and extend in parallel configuration through the entire length of a chloroplast (Figs. 4, 10). In the chloroplast proper, however, there are usually two more bands than in the pyrenoid matrix (Figs. 4, 10). The interband space in the chloroplast proper is narrower (20–60 nm) than in the pyrenoid region. Interthylakoid spaces in a band are very uniform throughout the entire length of a chloroplast (Fig. 6). The switching of thylakoids from one band to another and the fusion of two bands for a short distance are the features seen often in the chloroplast proper.

Thylakoid bands become indistinct near the distal region of chloroplast. Although the ultrastructural details have not been clearly determined yet, it is seen from Figs. 4, 6 and 10 that thylakoid membranes abruptly disappear in profile near the very distal region of the chloroplast. However, it is not true in another section; in Fig. 7, the thylakoid bands are clearly seen even at the very distal part while they are indistinct in the proximal region. On the basis of this evidence we presume that thylakoid membranes might shift their orientation by approximately 90°.

Osmiophilic granules are situated between thylakoid bands throughout the chlo-

roplast (Figs. 2, 4, 10), but usually at or near the transition zone from the pyrenoid area to the chloroplast proper (Figs. 4, 10). In some cases, they are also found in the central part or near the distal margin of pyrenoid. Any other kind of synthetic products was not found in the chloroplasts.

The chloroplast envelope is composed of two membranes (Figs. 5, 10). The chloroplast is always surrounded with a superimposed layer of endoplasmic reticulum (chloroplast-ER) (Figs. 5, 10). In the space between the Ch-ER and the chloroplast envelope along the pyrenoid region, there is a third membranaceous element which is ER-like structure. The ground substance and the membrane of this structure seem very similar in electron density to their surrounding material, so that it is not easy to identify the profile of this structure from the membranes of Ch-ER and chloroplast envelope (Figs. 5, 10). Direct association of this cisterna with Ch-ER or the outer membrane of the chloroplast envelope was not detected. The outer membrane of Ch-ER is, as seen in Figs. 8, 9, the extension of the outer nuclear membrane. One to several connecting points (Fig. 9) often occur between a nucleus and chloroplast because of fusion between the Ch-ER and nuclear envelope. Intermembrane space of the chloroplast envelope is very regular over the entire chloroplast surface and it is narrower than that of Ch-ER or that of thylakoid. Ch-ER is closely bordered to the chloroplast envelope around the chloroplast proper (Figs. 6, 10), but is separated by a space from the envelope around the pyrenoid (Fig. 10).

Discussion

Recent studies on algal taxa from the viewpoints of ultrastructure as well as biochemistry of photosynthetic pigments have shown great similarities. Indeed, it is possible to determine the organisms' systematic position at high taxonomic ranks, such as division (phylum) or class, if we refer exclusively to such informa-

tion (cf. MASSALSKI and LEEDALE 1969, HIBBERD and LEEDALE 1971, HIBBERD 1976, EDWARD 1976). In the following discussion, we will compare several ultrastructural features of *Pseudodichotomosiphon constrictus* with those of certain related taxa.

The present study has revealed at least the following three features on the chloroplast structure to be useful for elucidating the systematic position of *P. constrictus*; 1) the chloroplast has typical three-thylakoid bands, 2) no reduction of thylakoid number occurs in pyrenoid region relative to the chloroplast proper, and 3) the chloroplast is encircled by the extension of the outer nuclear membrane. These characteristics of the chloroplast would reveal that *P. constrictus* has some characters shared with the Xanthophyceae and Chrysophyceae, all belonging to the Chromophyta. Of these two classes, the latter is not known to accommodate such a taxon as having multinucleate and siphonous algae, such as *Vaucheria*.

The present study also reveals further differences between *P. constrictus* and most members of the Xanthophyceae, though they are not major.

The profile of a chloroplast in this alga is prominent in its peculiar morphology. As far as we know, such a shape of chloroplast has not been observed in any Xanthophycean algae.

Peripheral thylakoid has often been received as characteristic feature of the Xanthophyceae (e.g. DODGE 1973). Indeed, it has been found in most of the species ever having been examined in the Xanthophyceae, including certain species of *Vaucheria* (DESCOMPS 1963 b, OTT and BROWN 1974, MARCHANT 1972) and *Tribonema* (LEFORT 1962, FALK and KLEINIG 1968), *Botrydium granulatum* (FALK 1967) and *Mischococcus sphaerocephalus* (HIBBERD and LEEDALE 1971). However, there are two species known to have no such a thylakoid in this class: *Bumilleria sicula* (MASSALSKI and LEEDALE 1969) and *Bumilleriopsis filiformis* (HIBBERD and LEEDALE 1971). The present alga also lacks a peripheral thylakoid in the chloro-

plasts. This is the third record of a chloroplast without peripheral thylakoid known thus far in the xanthophycean algae.

The pyrenoids of *P. constrictus* are traversed by parallel three-thylakoid bands. This feature is in disagreement with that of *Vaucheria*. In *Vaucheria*, the thylakoid bands are not in parallel, but irregularly traversing through within the pyrenoid matrix (DESCOMPS 1963 a, b), or pyrenoid is a projecting type free of thylakoid (MARCHANT 1971).

Taking into consideration the similarities of gross morphology and essential features of cell components, it would be more natural to place *Pseudodichotomosiphon constrictus* in the Xanthophyceae of the Chromophyta, treating the genus as the autonomous taxon and classifying it next to the genus *Vaucheria*. This conclusion is also supported by our another work on the pigment analysis of *P. constrictus* (YOKOHAMA *et al.* in prep.), which will be described in the separating paper.

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References

- DESCOMPS, S. 1963 a. Contribution à l'étude infrastructurale des *Vaucheries* (Xanthophycées, Chromophytes). C. R. Acad. Sc. Paris **256**: 1333-1335.
- DESCOMPS, S. 1963 b. Observations sur l'infrastructure de l'enveloppe des chloroplastes de *Vaucheria* (Xanthophycées). C. R. Acad. Sc. Paris **257**: 727-729.
- DODGE, J. D. 1973. The fine structure of algal cells. Academic Press, London & New York.
- EDWARDS, P. 1976. A classification of plants into higher taxa based on cytological and biochemical criteria. Taxon **25**: 529-542.
- FALK, H. 1967. Zum Feinbau von *Botrydium granulatum* GREV. (Xanthophyceae). Arch. Mikrobiol. **58**: 212-227.
- FALK, H. and KLEINIG, H. 1968. Feinbau und Carotinoide von *Tribonema* (Xanthophyceae). Arch. Mikrobiol. **61**: 347-362.
- FOTT, B. 1971. Algenkunde. Gustav Fischer, Stuttgart.
- FRITSCH, F. E. 1935. The structure and reproduction of the algae. Vol. 1, Cambridge Univ. Press, Cambridge.
- HIBBERD, D. J. 1976. The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): a survey with some new observations on the ultrastructure of the Chrysophyceae. Bot. J. Linn. Soc. **72**: 55-80.
- HIBBERD, D. J. and LEEDALE, G. F. 1971. Cytology and ultrastructure of the Xanthophyceae. II. The zoospore and vegetative cell of coccoid forms, with special reference to *Ophiocytium majus* NAEGELI. Br. phycol. J. **6**: 1-23.
- HIROSE, H. 1972. General phycology. Uchida Rokakuho, Tokyo.
- HORI, T. and ENOMOTO, S. 1978. Electron microscope observations on the nuclear division in *Valonia ventricosa* (Chlorophyceae, Siphonocladales). Phycologia **17**: 133-142.
- LEFORT, M. 1962. Contribution à l'étude infra-microscopique du *Tribonema aequale* (PASCHE). C. R. Acad. Sc. Paris **254**: 3022-3024.
- LUTHER, H. 1953. *Vaucheria scheleicheri* DE WILD. neu für Nordeuropa. Mem. Soc. Fauna Flora Fennica **28**: 32-40.
- MARCHANT, H. J. 1972. Pyrenoids of *Vaucheria woroniniana* HEERING. Br. phycol. J. **7**: 81-84.
- MASSALSKI, A. and LEEDALE, G. F. 1969. Cytology and ultrastructure of the Xanthophyceae. I. Comparative morphology of the zoospores of *Bumilleria sicula* BORZI and *Tribonema vulgare* PASCHER. Br. phycol. J. **4**: 159-180.
- MCLACHLAN, J. 1973. Growth media—marine. In J. STEIN (ed.) Handbook of phycological methods. Culture methods and growth measurements. Cambridge Univ. Press, Cambridge.
- OKAMURA, K. 1936. Nippon Kaiso-shi. Uchida Rokakuho, Tokyo.
- OTT, D. W. and BROWN, Jr. R. M. 1974. Developmental cytology of the genus *Vaucheria* II. Sporogenesis in *V. fontinalis* (L.) CHRI-

- STENSEN. Br. phycol. J. 9: 333-351.
- REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.
- SEGAWA, S. 1956. Colored illustrations of the seaweeds of Japan. Hoiku-sha, Osaka.
- SEGAWA, S. and KAMURA, S. 1960. Marine flora of Ryukyu Islands. Extent. Serv., Univ. Ryukyus.
- TSENG, C. K. 1936. Studies of the marine Chlorophyceae from Hainan. Amoy Mar. Biol. Bull. 1: 129-200.
- YAMADA, Y. 1932. Note on some Japanese algae III. J. Fac. Sci. Hokkaido Imp. Univ. Ser. 5. 1: 109-123.
- YAMADA, Y. 1934. The marine Chlorophyceae from Ryukyu. J. Fac. Sci. Hokkaido Imp. Univ. Ser. 5. 3: 33-88.
- YAMAGISHI, T. 1964. Observation on some siphonous algae collected from Okinawa. J. Jap. Bot. 39: 82-90.
- YOKOHAMA, Y., KOBARA, T. and CHIHARA, M. (in prep.) Plastid pigments of *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus.

堀 輝三・高原隆明・千原光雄：クビレミドロの微細構造と
分類上の位置について

本研究によって、クビレミドロ (*Pseudodichotomosiphon constrictus*) の葉緑体が、1) 3枚のチラコイドよりなるバンドをもつ、2) ピレノイド域内において1バンド当りのチラコイド数は減少しない、3) 葉緑体-ERを有する、等の微細構造的特徴を示すことが明らかになった。これらの特徴は黄緑藻で知られたそれと基本的に一致する。(300-31 茨城県新治郡桜村天王台 1-1-1, 筑波大学生物科学系)

- Fig. 2. Longitudinal section of the siphonous frond showing cell organization. $\times 7000$.
- Fig. 3. Light micrograph of chloroplast viewed from frond surface.
- Fig. 4. Cross section of the frond showing peculiar profiles of chloroplasts. The proximal portion of chloroplast with a bulging pyrenoid is directed toward the central vacuole and the distal, grip-end like portion are against the cell wall. In chloroplasts many spherical granules with high electron density are prominent at or near the distal region of the pyrenoid matrix and in the chloroplast proper. $\times 7100$.
- Fig. 5. Proximal portion of pyrenoid showing that convergence of thylakoid-bands in a row at a most proximal point of chloroplast (arrow). Seven bands, each consisting of three thylakoids, are separated by a certain distance all over the band, and two of which terminate before reaching a proximal point. Outside the double membraned chloroplast envelope (triple arrowhead) is a superimposed chloroplast-ER (single arrowhead), and in the place between them tubular elements are present (double arrowhead). $\times 32500$.
- Fig. 6. Part of distal portion of chloroplast through which eight or nine three-thylakoid bands run. In the more distal end thylakoid bands become indistinct probably because of shift of thylakoid orientation. $\times 26500$.
- Fig. 7. In contrast to Fig. 6 the individuality of thylakoid membranes is clearly seen at the most distal end, but those at the proximal portion are indistinct. $\times 36300$.
- Fig. 8. Outer membrane of the nuclear envelope is continuous with that of Ch-ER (arrow). $\times 23000$.

- Fig. 9. Several connecting points between the chloroplast envelope and Ch-ER (arrows) and localized patches containing cytoplasm are seen. $\times 32700$.
- Fig. 10. Cross section of a chloroplast. A superimposed Ch-ER (single arrowhead) completely surrounds the chloroplast envelope (triple arrowhead) and between them a tubular-like elements (double arrowhead) lies, especially around the pyrenoid area. Around the chloroplast proper, Ch-ER is tightly adressed to the chloroplast envelope. $\times 23900$.

Abbreviations; C, chloroplast; CW, cell wall; M, mitochondrion; N, nucleus; Nu, nucleolus; P, pyrenoid.

