植物研究雑誌
J. Jpn. Bot.
67: 338–341 (1992)

Phycobionts of *Diploschistes diacapsis* (Lichenes)[1)]

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地衣類 *Diploschistes diacapsis* の共生藻

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(Received on May 9, 1992)

The phycobiont of *Diploschistes diacapsis* (Ach.) Lumbsch in Japan was identified as *Trebouxia excentrica,* which is the first record from the genus *Diploschistes.*

Diploschistes diacapsis (Ach.) Lumbsch is a crustose lichen growing on soil and rock surface. Friedl and Gärtner (1988) isolated *Trebouxia asymmetrica* and *T. showmanii* as phycobionts from the European specimens of this species. Recently, we collected a fresh specimen of *D. diacapsis* in western Japan and isolated phycobionts from it. In the present paper, we gave a results of taxonomic studies of the phycobiont.

Materials and Methods

A fresh specimen of *Diploschistes diacapsis* (T. Okamoto-2694, HIRO) was collected at about 180 m alt., Mt. Shiraki, Hiroshima-shi, western Japan (Fig. 1).

The phycobiont was isolated from a fresh lichen thallus by the following procedure. Small pieces of washed thallus of *D. diacapsis* were ground between two glass slides. The suspension of phycobiont cells mixed with mycobiont hyphae were spread on Bold's Basal Medium (BBM) agar plates as modified by Bischoff and Bold (1963), and cultured under standard conditions (20°C, 2000 lux, 12 h light/12 h dark cycles). After about one month, colonies of phycobionts were visible and they were transferred to BBM agar slants. Axenic strains were obtained by the micropipette method (Ahmadjian 1967). Then they were cultured on BBM agar slants under standard conditions for two to four weeks to observe their morphological characters and life cycles. Light microscopy was carried out using a Nikon XF Microscope. A dilute aqueous methylene blue solution was used to determine the presence of gelatinous matrices, and an Azocarmin G solution was used for observations of pyrenoids. Algal strains used in this study are

deposited in the Botanical Institute, Hiroshima University (CCHU).

Results and Discussion

Four strains of unicellular green algae were isolated from a thallus of *D. diacapsis*. As they showed the morphological characters of the genus *Trebouxia*, they were compared with the descriptions and figures by Archibald (1975), Hildreth and Ahmadjian (1981) and Gärtner (1985). They are also compared with *Trebouxia* strains obtained from the Culture Collection of Algae at the University of Texas (UTEX). The characteristics of all isolated phycobionts showed that they are identical with those of *Trebouxia excentrica* Archibald.

Trebouxia excentrica Archibald, Phycologia **14:** 128 (1975)

In log phase cultures, young vegetative cells spherical to subspherical. Most mature vegetative cells spherical, 15–18 μm in diameter (Fig. 2, A–C). Chloroplast of each cell deeply incised, and with pyrenoid without starch sheath. The pyrenoid at the excentric position. Cell walls thin, about 0.3–0.5 μm in thickness. Vegetative cells without gelatinous matrices. Zoospores and aplanospores are observed, but autospores not observed. Zoospores pear-shaped, 7 μm in length, 3–3.5 μm in width, with two flagella of equal length at the anterior end and a minute stigma in the anterior part of chloroplast (Fig. 2, F). Contractile vacuoles present. Zoospores move rapidly and becoming spherical vegetative cells after the loose of their mobility and flagella. Aplanospores spherical, 3–5 μm in diameter. Sixty-four or 128 aplanospores produced in each sporangium (Fig. 2, D). Sexual reproduction not observed.

Culture number: 5615 (CCHU)

Trebouxia excentrica was first isolated from *Stereocaulon dactylophyllum* var. *occidentale* (Archibald 1975). It has been also isolated from *Cladonia bacillaris, C. leporina, Huilia tuberculosa, Lecidea metzleri* and *Lepraria* sp. (Hildreth and Ahmadjian 1981) and also from *Evernia divaricata, E. prunastri, Usnea florida, U. dasypoga* and *U. rigida* (Meisch 1981). In Japan,

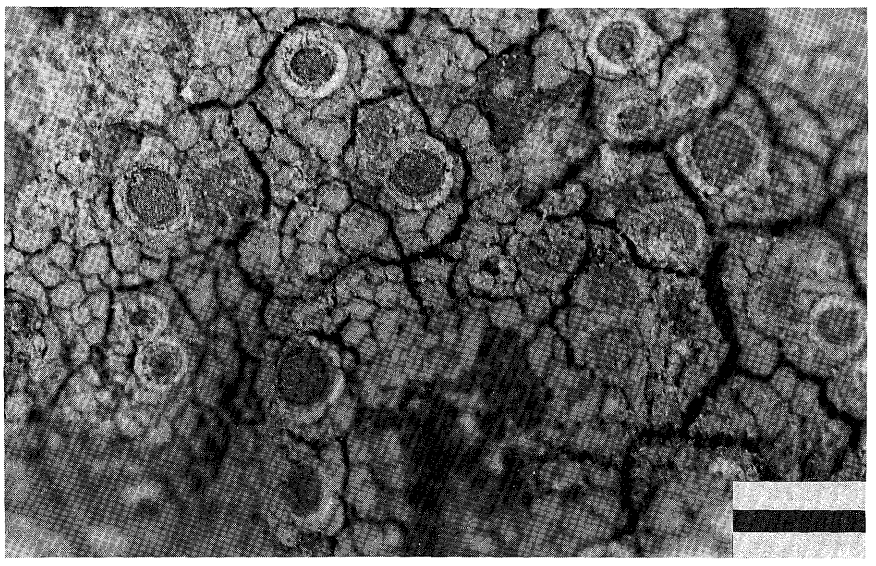

Fig. 1. *Diploschistes diacapsis* (Ach.) Lumbsch. Scale = 1 mm.

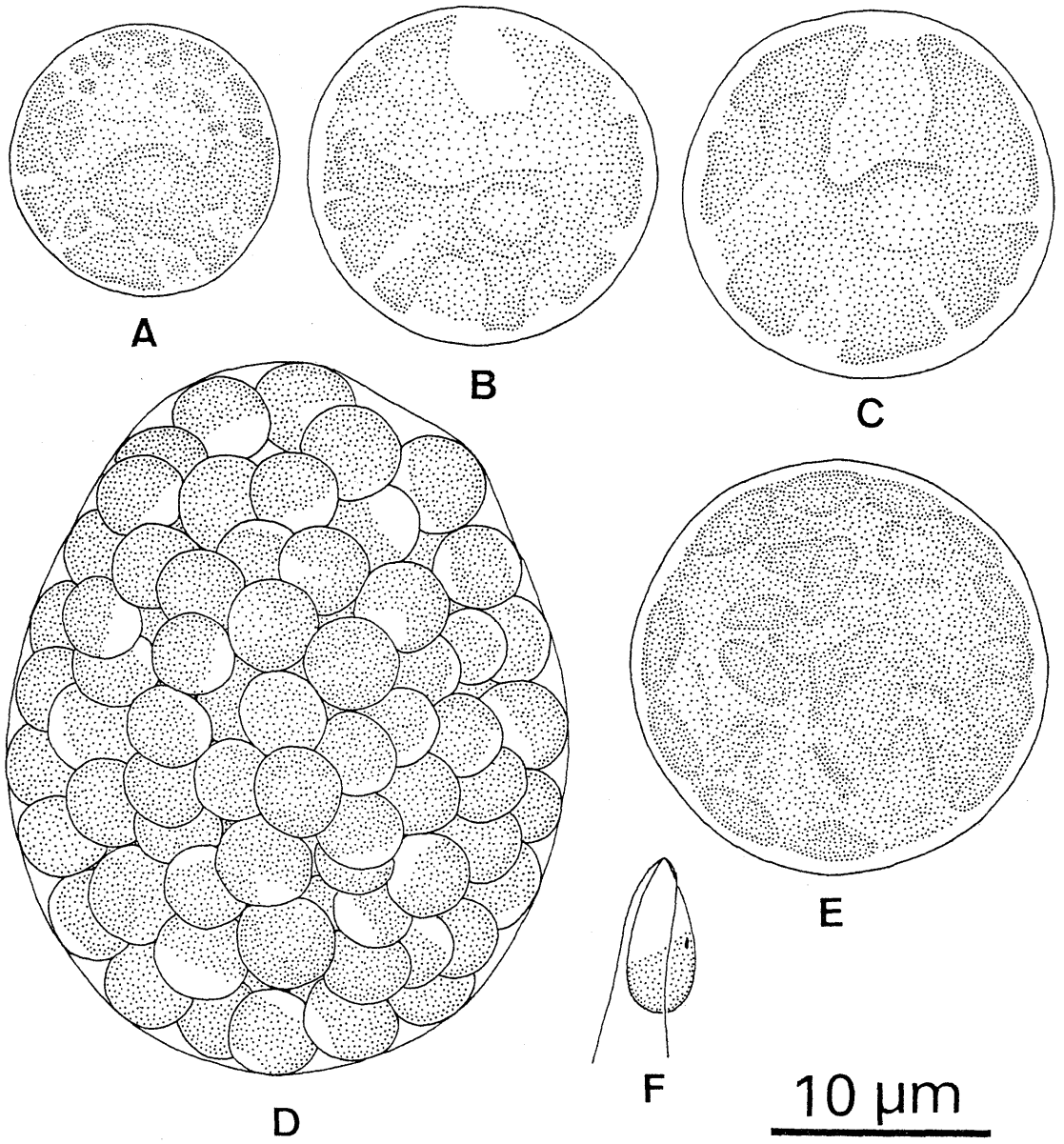

Fig. 2. *Trebouxia excentrica* Archibald. A–C, Vegetative cells. D, Aplanosporangium. E, Zoosporangium. F, Zoospore.

Yoshimura et al. (1987) reported it as the phycobiont of *Cladonia vulcani*.

Phycobionts of the genus *Diploschistes* were reported by Friedl (1987) and Friedl and Gärtner (1988) based on the eight European specimens. They are *Trebouxia asymmetrica, T. crenulata, T. gigantea, T. irregularis* and *T. showmanii*. Friedl and Gärtner (1988) reported *Trebouxia asymmetrica* and *T. showmanii* as the phycobionts of *Diploschistes diacapsis* (reported under *D. albescens* or *D. steppicus*). Therefore, *T. excentrica* is the third species so far described as the phycobiont of *D. diacapsis* and is the first record from this lichen genus. This fact suggests that *D. diacap-*

sis may have more than three species of phycobionts. We had some reports as the similar facts above-mentioned. For instances, Uyenco (1965) reported four species of *Trentepohlia* as phycobionts of *Coenogonium interplexum* and Takeshita et al. (1991) also recently reported *T. erici* and *T. glomerata* as the phycobionts of Japanese *Cladia aggregata*. These facts seem to show the diversity of symbiotic relationship between some species of lichens and their phycobionts.

We wish to express our thanks to Dr. H. Kashiwadani of National Science Museum, Tokyo, for his valuable advice on the manuscript. Thanks are also due to Dr. J. M. Glime of Michigan Technological University for the correction of the English text.

Endnote

[1)]Contribution from the Phytotaxonomical and Geobotanical Laboratory, Hiroshima University, N. Ser. No. 428.

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要 旨

広島県白木山で採集したキッコウゴケ属の地衣類, *Diploschistes diacapsis* (Ach.) Lumbsch から共生藻を分離・培養し, 分類学的検討を行った. その結果, 共生藻は緑藻類の *Trebouxia excentrica* Archibald であることが確認された. 本種がキッコウゴケ属の地衣類から分離されたのは初めてである.