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Observations on the type of *Liagorophila endophytica* YAMADA (Acrochaetiaceae, Rhodophyta)[1)]

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The lectotype (SAP 20646) specimen of *Liagorophila endophytica* YAMADA was examined to know exact characteristics of the species. Plants are endophytic in *Liagora orientalis* J. AGARDH, composed of creeping filaments and erect ones, with a stellate chloroplast per cell, and produce monosporangia. Fertilized carpogonia divide longitudinally. Gonimoblast filaments develop horizontally and perpendicularly to the axis of the carpogonium. Carposporangia are produced on the upper side of the cells of the gonimoblast filaments.

The occurrence of the species in Hachijo Island, Japan, was newly recorded by an examination of the specimens of *Helminthocladia australis* HARVEY collected by Y. YAMADA. The relationship was discussed between the results from the present examination and previous records relevant to the species.

Key Index Words: *Acrochaetiaceae*; Liagorophila; L. endophytica; *reproduction*; *Rhodophyta*; *taxonomy*.

The Acrochaetiaceae has long been an object of much argument concerning the generic circumscription. Five or six taxonomic systems for generic definition are presented in the family (PAPENFUSS 1945, 1947, KYLIN 1956, FELDMANN 1962, WOELKERLING 1971, DIXON and IRVINE 1977, GARBARY 1979, STEGENGA 1979). The disagreement among the proposed systems may not be easily solved because almost the same set of data is available for each opinion (see also STEGENGA 1979). As none of the systems is exclusively approved by most phycologists, some confusion may continue in the use of binomials of the members of the Acrochaetiaceae. LEE (1980) has given a clue to solve partly the systematic problem.

Liagorophila was established by YAMADA (1944) with an acrochaetioid alga *L. endophytica*; an endophyte in *Liagora orientalis* J. AGARDH collected at Daibanratu, Formosa. He characterized *Liagorophila* as "…by the structure of the carpogonial branches, and by the development of the cystocarps". He described in details carpogonia and post-fertilization development of the alga in his previous paper where he thought the carpogonia as the female structure of the host (YAMADA 1938: fig. 1, D-H). Thereafter, FAN and LI (1964) reported the species from Hainan Island, China. ABBOTT (1966) also reported the species occurring in Tanegashima Island, Japan. However, some characteristics of the plants from the two localities unfortunately contradicted each other. The disagreement of descriptions between the latter two plants arose some confusion in the specific as well as in the generic circum-

1) This paper is dedicated to Professor R. F. SCAGEL, Department of Botany, University of British Columbia, Canada, on the occasion of his retirement.

scription of *Liagorophila*.

The purpose of this study is to furnish details on the characteristics of *L. endophytica* with the lectotype material and allied specimens, and to discuss the plants from the two localities, Hainan Island and Tanegashima Island.

Materials and Methods

Three materials of *L. endophytica* collected by Y. YAMADA were examined. These are represented here by the name of host species as follows; 1) *Liagora orientalis* J. AGARDH, Daibanratu (Formosa), March 1924 (SAPO 20646); 2) *Helminthocladia australis* HARVEY, Hachijo Island (Japan), 19th of July 1951 (SAPO 47546); 3) *Helminthocladia australis* HARVEY, Hachijo Island (Japan), 17th of July 1951. The first and the second materials are dried herbarium specimens, while the third one is microscopic slides. The morphological description in this paper is mainly based on the third material identified by Y. YAMADA.

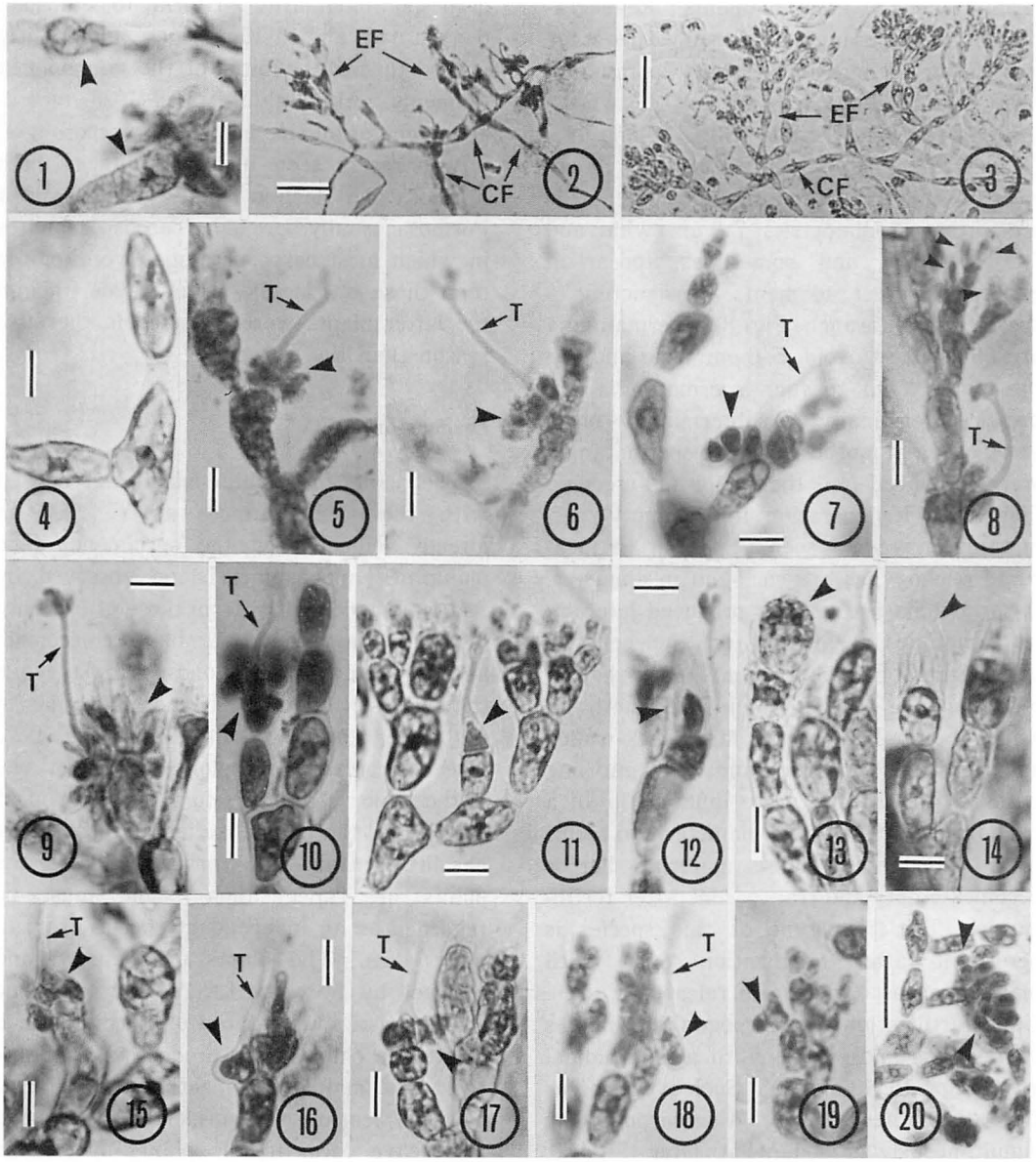
For microscopic observation small pieces of the host thallus of the dried herbarium specimens were taken off and soaked in 10% aqueous sodium carbonate for 1-4 hrs (WOELKERLING 1970) and they were stained with 0.5% (w/v) cotton blue in lactic acid/phenol /glycerol/water (1 : 1 : 1 : 1) solution and mounted in 50% glycerol-seawater on microscopic slides. The material of *Liagora* was decalcified in 5% aqueous hydrochloric acid for 30-60 min before staining.

Results

Most part of the thallus of *Liagorophila endophytica* weaves among the assimilatory filaments (cortical layer) of the hosts, and gives rise to erect filaments toward the host surface. The thallus shows the heterotrichous habit composed of creeping and erect filaments (Figs. 2 and 3). The creeping filaments are sinuous and undulated, and issue branches like themselves resulting in a complicated network of the filaments in the cortical layer of the host. However, no creeping filaments penetrate into the medullary layer or protrude beyond the surface of the host. Occasionally a part of the creeping filaments approaches the host surface and produces sessile carpogonia or monosporangia as well as short (one- or two-celled) erect filaments. Cells of creeping filaments are fusiform when they do not issue branches, 10-15 μm wide and 30-45 μm long. If a cell issues branches, it usually protrudes toward the branches showing a T-shape or a triangular shape.

Erect filaments usually consist of three to five cells. The erect filament branches dichotomously or trichotomously from the distal part of its component cell. The apex of erect filaments comes to lie on a plane parallel to the tips of the assimilatory filaments of the host. When a cell issues branches, there is no protrusion of the cell. Even if there is, the protrusion is less prominent than those in creeping filaments. The cells of erect filaments are gradually getting shorter toward the tips of erect filaments, pyriform to globose in shape, 8-13 μm wide and 9-35 μm long. Chloroplasts are stellate with a central pyrenoid (Figs. 1 and 4). There is no difference in the chloroplast morphology between creeping filaments and erect ones. Hair or hair-like structures were not observed. However, short bar-like structures measuring 5-6 μm thick and up to 30 μm long were often encountered on the terminal cell of erect filaments. The bar-like structure is sometimes produced by means of regeneration in an empty monosporangium. Although no chloroplast was confirmed in the structure, it is not hyaline as is the hairs of acrochaetioid algae.

Monosporangia (Fig. 13) are terminal, solitary or rarely in pairs on a cell of erect filaments, obovate to globose in shape, 11-15 μm wide and 15-18 μm long with a stellate chloroplast. It is not so easy to discern monosporangia because of their morphological similarity to vegetative cells. Empty monosporangia are quite common on the



Figs. 1–20. *Liagorophila endophytica* YAMADA. All figures are drawn from the plants collected in Hachijo Island unless otherwise indicated. CF, creeping filament; EF, erect filament; T, trichogyne. Scale bar in Figs. 2 and 3 (50 μm); in Fig. 20 (30 μm); in the others (10 μm).

1. Stellate chloroplast (arrow heads) of the lectotype. 2. Thallus habit of the lectotype. 3. Thallus habit. 4. Stellate chloroplast. 5. Gonimoblast filament in development (arrow head) of the lectotype. 6. Postfertilization development (arrow head) of the lectotype. 7, 15 and 17. Postfertilization development (arrow heads). 8. Spermatangial branch bearing spermatangia (arrow heads) of the lectotype. 9. Older carposporophyte showing most carposporangia being empty (arrow head) of the lectotype. 10. Postfertilization development somewhat advanced (arrow head). 11. Thallus showing a monoecious form with a carpogonium (arrow head) and many spermatangia on the terminal cells of erect filaments. 12. Regeneration in an empty monosporangium (arrow head) of the lectotype. 13. Monosporangium. 14. Empty monosporangium showing a successive formation of sporangium in an old membrane. 16 and 18. Fertilized carpogonia being inflated (arrow head). 19. Longitudinal division of a fertilized carpogonium (arrow head). 20. Mature carposporophyte. Note carposporangia on the cells of gonimoblast filaments (arrow heads).

terminal cells of erect filaments. Regeneration of monosporangia is presumed to occur two or three times in an empty sporangium from the frequent appearance of two- or three-folded empty walls (Figs. 12 and 14).

The plants of *L. endophytica* are monoecious (Fig. 11). Spermatangial mother cells are rather isodiametric, 7-9 μm wide and 7-10 μm long, and sometimes appear in groups on erect filaments constructing a spermatangial branch (Fig. 8). Spermatangia are ellipsoid to ovoid, 5-6 μm wide and 6-8 μm long. Two to four spermatangia are produced terminally or subterminally on a spermatangial mother cell. No spermatangia were produced on the cells of creeping filaments. Regeneration in an empty spermatangium was not seen. Spermatia attached to trichogynes are ca. 5 μm in diameter.

Carpogonia are usually produced laterally or sometimes terminally on the cells of upper parts of erect filaments, flask-shaped, 5-10 μm wide and 10-13 μm long with an acute apex. Trichogynes are thick-walled and rigid, ca. 2 μm wide and 18-30 μm long with an inflated tip. The inflated tip of a trichogyne is 5-6 μm in diameter and seems to be filled with dense material, but it becomes empty and transparent after fertilization. The trichogyne of this species is persistent after fertilization even until mature carposporangia are released (Fig. 9). Upon fertilization the carpogonium inflates on one side and divides with a longitudinal wall (Figs. 16, 18 and 19). Such events occur from the other sides of the carpogonium simultaneously or somewhat later (Figs. 6, 7, 15 and 17). As a result, two to four cells are produced from the carpogonium (Figs. 5 and 10). Each cell becomes the origin of a few-celled gonimoblast filament. The cell of gonimoblast filaments is small and various in shape, measuring 5-7 μm in diameter. The gonimoblast filaments develop perpendicularly to the axis of the carpogonium. Each cell of the gonimoblast filament produces one to three carposporangia unilaterally on the upper side of the cells of the gonimoblast filaments (Fig. 20). The carposporangia are pyriform to ellipsoid, 6-8 μm wide and 13-15 μm long, and slightly curved toward the initial of the gonimoblast filaments. Although YAMADA (1938) recorded regeneration in empty carposporangia, it was rarely seen in the plants of both Formosa and Hachijo Island. The plants of Formosa usually bore older carposporophytes in which most carposporangia were empty than those of Hachijo Island, even though the latter plants were collected in the later season than the former.

Discussion

The plants of Hachijo Island agree well with those of Formosa as Y. YAMADA already indicated on the specimens. The plants of Tanegashima Island observed by ABBOTT (1966) differ from those of Formosa in having a parietal laminate chloroplast and in producing no monosporangia while the thallus habit of the former is similar to the latter (see ABBOTT 1966: Figs. 1-3). The chloroplast morphology has been one of the major characters for identification of species in the Acrochaetiaceae because it has little intraspecific variation. Thus, the plants of Tanegashima Island must be regarded as an independent species from *L. endophytica*. The plants of Hainan Island reported by FAN and LI (1964) shares some characteristics with those of Formosa, such as stellate chloroplasts, production of monosporangia, and postfertilization development. No cells bearing a monosporangium showed a concave form in the plants of Hachijo Island as do the plants of Hainan Island. FAN and LI (1964) reported the occurrence of active regeneration in empty carposporangia. It also seems to us that the plants of Hainan Island are hardly regarded as *L. endophytica* in view of the thallus habit, the shape of monosporangial mother cells, and the sexuality (see also FAN and LI 1964, ABBOTT 1966, GARBARY 1980). Thus, it is inferred that more species may be attributed to *Liagorophila*. *L. endophytica* also differs from *Auduinella imitator* (ABBOTT) GARBARY

Table 1. Comparison of some characteristics of the plants from each locality. fil. = filaments, W/L = width/length.

Locality	Daibanratu Formosa	Hainan Isl. China	Tanegashima Isl. Japan	Hachijo Isl. Japan
Host	*Liagora orientalis*	*Liagora* sp.	*Liagora tanakae*	*Helminthocladia australis*
Thallus	creeping and erect fil.	creeping fil. only	creeping and erect fil.	creeping and erect fil.
Cell form	long doliiform	triangular	fusiform	fusiform
Cell size (W/L)	16 μm/16-32 μm	8-10 μm/10-22 μm	10-15 μm/25-35 μm	10-15 μm/30-45 μm
Chloroplast	stellate	stellate	laminate	stellate
Monosporangia	present	present	absent	present
Sexuality	monoecious	dioecious*	monoecious	monoecious
Pyrenoid	present	?	present	present
References	YAMADA (1938) YAMADA (1944) SAPO 20646	FAN and LI (1964)	ABBOTT (1966)	present study

* Occasionally monoecious.

in chloroplast morphology and postfertilization development of a carpogonium (ABBOTT 1962). It needs further study for the critical comparison among *L. endophytica*, *A. imitator* and *A. liagorae* (BØRGESEN) WOELKERLING as well as the plants of Hainan Island and Tanegashima Island. Several characteristics of the plants from Hainan Island, Tanegashima Island, and Hachijo Island are compared with the lectotype specimen (SAPO 20646) in Table 1.

KYLIN (1956) and FELDMANN (1962) recognized the genus *Liagorophila*. WOELKERLING (1971) regarded *Liagorophila* as a doubtfully distinct genus, emphasizing the necessity of further study. STEGENGA (1979) and WOELKERLING (1983) placed stress on the necessity of caution for having an outspoken opinion on the validity of distinguishing *Liagorophila* without observation of the plants relevant to the genus by themselves. LEE and KUROGI (1978) preferred to use the original valid generic epithet for *Rhodochorton subimmersum* SETCHELL et GARDNER until a generally recognized taxonomic system is provided in the Acrochaetiaceae. However, GARBARY (1980) synonymized *Liagorophila* under *Auduinella*, proposing a new name *A. yamadae* GARBARY for the type species of the former genus (see also GARBARY 1979). He pointed out that the use of the characteristics based on the development of a fertilized carpogonium for generic circumscription will be difficult until sexual reproduction is demonstrated in most species of the Acrochaetiaceae. We also think that at present the information on sexual reproduction has been provided in too few species to be based on the generic circumscription in the family. We prefer to maintain *Liagorophila* as a distinct genus and characterize the genus by the production of monosporangia, the longitudinal division of a fertilized carpogonium, the horizontal development of gonimoblast filaments, and the production of carposporangia on the upper side of the cells of the gonimoblast filaments.

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李 龍弼*・吉田忠生・黒木宗尚**: *Liagorophila endophytica* に関する観察**

Liagorophila endophytica YAMADA の選定タイプ標本 (SAP 020646) について観察を行なった。この植物はフサコナハダ *Liagora orientalis* に内生し，ほふく枝と直立枝よりなり，細胞には1個の星形色素体を含み，単胞子を形成する。受精した造果器は縦の膜で分裂し，造胞糸は横に拡がってその上に果胞子嚢をつける。

この種が八丈島産のベニモズクからも観察された。この種に関する最近の報告についても言及した。(* 590 大韓民国済州市 済州大学校生物学科 ** 060 札幌市北区北十条西8丁目 北海道大学理学部植物学教室)