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Morphological study of *Callithamnion callophyllidicola* (Ceramiaceae, Rhodophyta) from the Oki Islands in the Sea of Japan[1]

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Callithamnion callophyllidicola Yamada was collected in abundance from the Oki Islands in the Sea of Japan. Its morphology was examined detailedly and is compared with previous reports made by Segawa (1942, 1949) and Kawashima (1960) on this species and that of its allied 3 species which have been assigned to the genus *Aglaothamnion,* such as *C. tripinnatum, C. decompositum* and *C. oosumiense* comb. nov. The result of this study suggests that the material used for Kawashima's study is probably a distinct species.

Introduction

Callithamnion callophyllidicola was described by Yamada (1932) on the basis of a collection from Enoshima, Kanagawa Prefecture on the Pacific coast of Honshu, and has been reported from Honshu coast, the Inland Sea and Kyushu in Japan as well as from various sites in Korean waters (Boo *et al.* 1989). It is apparently restricted to deep water and to shaded places in shallow water (Yamada 1932; Kajimura 1987).

Segawa (1942, 1949) reported his observations on procarp and early stages of carposporophyte development of this species based on material collected from Izu-Susaki, Arashidomari and the type locality. Later, Kawashima (1960) also reported his morphological observations on this species based on material collected from Matsushima Bay, Miyagi Prefecture and Ōma-Benten-Jima, Aomori Prefecture. However, the result of their studies differ from that of the present study in various ways and the results are precisely compared herein.

More than 17 species of *Aglaothamnion* have been reported from Mediterranean Sea, Adriatic Sea, Atlantic Ocean, Pacific Ocean and Indian Ocean (Abbott 1972; Børgesen 1945, 1952; Feldmann 1954; Feldmann-Mazoyer 1940; Halos 1965a, 1965b; Itono 1977; South and Tittley 1986) since the genus was established by Feldmann-Mazoyer (1940, P. 451) based upon *Aglaothamnion furcellariae* (J. Ag.) Feldmann-Mazoyer. Features used by Feldmann-Mazoyer (1940) to characterize the genus were the uninucleate condition of the cells, the arrangement of the carpogonial branch cells in

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[1] Contribution No. 49 from Oki Marine Biological Station, Shimane University.

zigzag or U-shaped fashion and the irregularly lobed gonimolobe. *Callithamnion callophyllidicola* which has all of those characters of *Aglaothamnion* would be trasferred to *Aglaothamnion,* on the other hand, Harris (1966) and Dixon and Price (1981) concluded that the genus *Callithamnion* should be conceived broadly to include species that were previously assigned to the genus *Aglaothamnion,* and that the latter should be considered as a later synonym of the former. Therefore, this entity is still treated as a species of *Callithamnion* in this study.

Segawa (1942) newly detected uninucleate condition of the vegetative cells of this species, but he did not made morphological comparison with those species previously assigned to *Aglaothamnion,* and it is made with 3 species of them in the present study.

Material and Methods

Material collected by the present writer from the Oki Islands and the holotype and isotype specimens (SAP 13082), both of which were mounted on one herbarium sheet, were used for this study. Many mature specimens were collected at low tide in shaded places in Kamo Bay and Takatori, Oki Islands, during the period between late September 1987 and early January 1989 (Table I).

Fresh specimens as well as specimens preserved in 10% formalin-seawater were used for this study. Whole specimens were stained with a 1% aqueous solution of aniline blue acidified with acetic acid (9 : 1 v/v) and mounted in a 50% aqueous solution of rice syrup acidified with acetic acid (33 : 1 v/v). Fresh and dried specimens used for cytological study were stained with acetocarmine (Sass 1966).

Table I. List of the collections of material.

No.	Date	Locality	Substratum	Maturity		
OS 10035	Sep. 22, 1987	Takatori	On *Cladophora ohkuboana* Holmes	♀		⊛
OS 10036	Dec. 7, 1987	Kamo Bay	On *Corallina pilulifera* Postels *et* Ruprecht, *Plocamium telfairiae* (Harvey) Kützing	♀		
OS 10037	Dec. 10, 1987	Kamo Bay	On *Callophyllis* sp., *Plocamium telfairiae* (Harvey) Kützing, *Erythroglossum minimum* Okamura	♀		
OS 10038	Jan. 4, 1989	Kamo Bay	On *Cladophora ohkuboana* Holmes, *Corallina pilulifera* Postels *et* Ruprecht	♀		⊛
OS 10039	Jan. 5, 1989	Kamo Bay	On *Cladophora ohkuboana* Holmes, *Codium fragile* (Suringar) Hariot	♀	♂	⊛
OS 10040	Jan. 6, 1989	Kamo Bay	On *Cladophora ohkuboana* Holmes, *Dictyota dichotoma* (Hudson) Lamouroux, *Corallina pilulifera* Postels *et* Ruprecht	♀	♂	⊛
OS 10041	Jan. 10, 1989	Kamo Bay	On *Cladophora ohkuboana* Holmes, *Corallina pilulifera* Postels *et* Ruprecht	♀		⊛

Observations

Callithamnion callophyllidicola Yamada 1932: 270. Segawa 1942: 206; 1949: 143. Kawashima 1960: 103. Kajimura 1987: 380.

Vegetative structure

The thallus is bright red, filamentous, monopodial in growth, up to 1 cm in height, composed of uninucleate cells (Figs. 6, 7), abundantly branched in one plane (Figs. 27–29) and lacks cortication. It is attached by primary basal rhizoids arising from lower cells of the main axis (Fig. 4) and adventitious rhizoids arising from the lower axial cells of whorl-branches (Fig. 5), or from cells of their branchlets. Both primary and adventitious rhizoids multicellular, dichotomously branched and terminated by a simple filament or a multicellular disc (Figs. 2, 4, 5, 20). The thallus is usually erect without prostrate branches but is sometimes heterotrichous (Fig. 2).

Apical cells of indeterminate axes divide obliquely, cutting off short cylindrical cells up to 7 μm in diameter by 13 μm long (Fig. 1). Axial cells in mature portions of the thallus may reach 30 μm in diameter and 100 μm in length. Laterals are initiated almost immediately below the apical cell and are arranged alternately on the axis. Some laterals continue to develop, thus forming indeterminate branches at irregular intervals along the axis. Whorl-branches are straight and bear 1–3 orders of pinnate branchlets in the plane of the thallus. Axial cells of indeterminate branches and whorl-branches are arranged in more or less zigzag fashion (Figs. 27–29), although branchlet cells are not so arranged. Branchlets are sometimes provided with unicellular terminal hairs of 78 μm or more in length (Fig. 3).

The basal cells of whorl-branches are usually pentagonal, elongate and with an abaxial branchlet. Apical cells of branchlets divide more or less obliquely, but sometimes transversely, cutting off short cylindrical derivatives 7 μm in diameter by 17 μm long. The branchlets are more or less curved either adaxially or abaxially. Gland cells are absent. Chromatophores are irregularly band-shaped at maturity (Fig. 8).

Reproductive structures

Procarp and carposporophyte

Female fertile segments are shorter than other segments, formed singly, and are restricted to axes of indeterminate branches (Fig. 9) throughout the thallus, except in the lower parts. Usually two opposite fertile pericentral cells (Fig. 10) are cut off from each fertile segment. One carpogonial branch is borne on the first-formed pericentral cell which thus acts as a supporting cell and is composed of four cells which are produced from the initial cell by sequential oblique divisions. The terminal carpogonium bears a straight trichogyne reaching 150 μm in length (Figs. 11–13). The four carpogonial branch cells are arranged in zigzag fashion (Fig. 13) and the carpogonial branch is directed towards the second fertile pericentral cell (Figs. 12, 13). Sterile cells are absent from the procarp.

Following presumed fertilization, the trichogyne degenerates and the supporting cell and the second fertile pericentral cell each cut off an auxiliary cell (Fig. 15). The carpogonium cuts off a secondary carpogonium by longitudinal division (Fig. 15). The two segments of the carpogonium thus formed each produce a connecting cell one of which fuses with each of the two auxiliary cells. Presumably a diploid nucleus is thus transferred to each auxiliary cell. The auxiliary cells then each divide to form a proximal foot cell and a distal gonimoblast initial which usually produces two gonimolobe initials one after the other. These gonimolobes also mature in sequence (Figs. 16, 18) and divide to form a number of cells which all, except a few elongate lower cells become carposporangia each ca. 26 μm in diameter. Mature gonimolobes are compressed, more or less lobed, cordate and 133–266×93–333 μm in size (Figs. 19, 27). No fusion cell was formed during the development of the carposporophyte and no involucre nor pericarp is developed.

Spermatangia

Male fertile segments are restricted to branchlets throughout the thallus except in the lower parts (Figs. 17, 28). One to two hemispherical initial cells of spermatangial mother cell branches arise adaxially on each male fertile segment. These divide successively several times in planes perpendicular to the branchlet surface to form an irregularly branched spermatangial mother cell branch which consists of a few to several elongate cells and extends toward the apex of the branchlet (Fig. 17). Each mother cell produces terminally 2–3 ellipsoidal spermatangia of 2.6–4×4–6.5 μm in size. Discharged spermatia are ca. 2.6 μm in diameter (Fig. 14).

Tetrasporangia and parasporangia

Tetrasporangia are sessile, subspherical to ellipsoidal, 23–50×17–66 μm in size,

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Figs. 1–10. *Callithamnion callophyllidicola* Yamada.

Fig. 1. Apical part of a frond showing the production of whorl-branches and division of apical cell of axis.

Fig. 2. Part of a frond showing a repent (arrows) and erect branches.

Fig. 3. Apex of a branchlet with pigmented unicellular terminal hair.

Fig. 4. Basal part of a frond showing basal rhizoids (arrows).

Fig. 5. Lower part of a frond showing adventitious rhizoids (arrows).

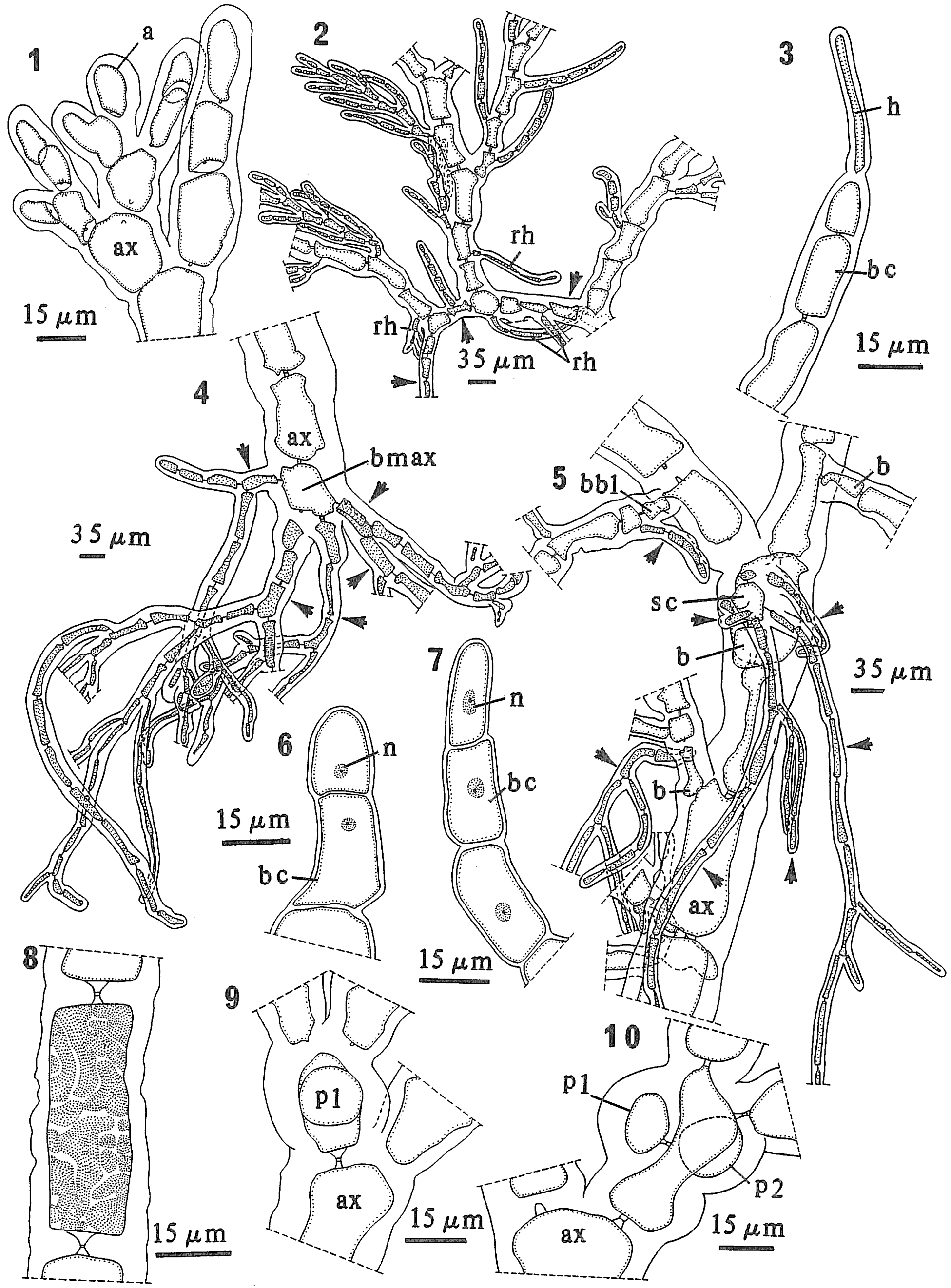
Fig. 6. Apical part of a branchlet of holotype specimen showing 2 uninucleate cells.

Fig. 7. Apical part of a branchlet of a specimen collected from the Oki Islands showing 3 uninucleate cells.

Fig. 8. Part of a branchlet showing chromatophores in a branchlet cell.

Fig. 9. Part of the main axis of a female plant showing a female fertile segment with a single fertile pericentral cell.

Fig. 10. Part of the main axis of a female plant showing a female fertile segment with an opposite pair of fertile pericentral cells.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tetrahedrally divided and borne singly or in pairs adaxially on the segment of branchlets throughout the thallus except the lower portion (Figs. 21, 22, 29).

Parasporangia are ca. 15 μm in diameter and formed subterminally on the branchlet of the tetrasporophyte (Fig. 23), which is an irregular mass of some sporangia similar to those in *C. decompositum* (Halos 1965a, Fig. 2, E).

Figs. 11–17. *Callithamnion callophyllidicola* Yamada.

Fig. 11. Part of the main axis of a female plant showing a young procarp with a 3-celled young carpogonial branch (cb1-cb3).

Fig. 12. Part of the main axis of a female plant showing a young procarp with a 4-celled young carpogonial branch directed towards the second pericentral cell (cb1-cb4) and with a young trichogyne (ytr).

Fig. 13. Part of the main axis of a female plant showing a procarp with a mature carpogonial branch (cb1-cb4) with a well developed trichogyne (tr).

Fig. 14. Apical part of a trichogyne with 2 spermatia attached to it.

Fig. 15. Part of the main axis of a mature female plant showing early development of the carposporophyte following fertilization of the procarp. The supporting cell and the second fertile pericentral cell each cut off an auxiliary cell (aux) opposite the carpogonial branch on the former and distally on the latter.

Fig. 16. Part of a mature female plant showing a carposporophyte at an early stage of development.

Fig. 17. Part of a mature male plant showing spermatangia (arrows).

Figs. 18–23. *Callithamnion callophyllidicola* Yamada.

Fig. 18. Part of a mature female plant showing a developing carposporophyte.

Fig. 19. Part of a mature female plant showing a mature carposporophyte.

Fig. 20. Part of a plant showing a terminal disc of a basal rhizoid.

Fig. 21. Part of a tetrasporophyte showing tetrasporangia at various stages of development and formed in the upper part of an indeterminate branch.

Fig. 22. Part of a tetrasporophyte showing 2 young and one mature tetrasporangia.

Fig. 23. Part of a tetrasporophyte showing a group of parasporangia (arrow).

Figs. 24–27. *Callithamnion callophyllidicola* Yamada.

Fig. 24. Holotype which is epiphytic on *Callophyllis crispata* and indicated "Type!" by Yamada.

Fig. 25. Isotype which is epiphytic on *Callophyllis crispata* and indicated "Cotype!" by Yamada.

Fig. 26. Isotype which is indicated "Cotype!" by Yamada.

Fig. 27. Part of a fresh specimen with carposporophyte, collected from Kamo Bay, the Oki Islands on January 5, 1989, showing 2 mature gonimolobes (arrows). Note branching in one plane and zigzag arrangement of axial cells.

Figs. 28, 29. *Callithamnion callophyllidicola* Yamada.

Fig. 28. Part of a mature male plant collected from Kamo Bay, the Oki Islands on January 5, 1989, branching in one plane and stained with aniline blue, showing spermatangial clusters (arrows).

Fig. 29. Part of a fresh mature tetrasporophyte collected from Kamo Bay, the Oki Islands on January 5, 1989, branching in one plane, showing distichous branching of the thallus and many tetrasporangia at various stages of development.

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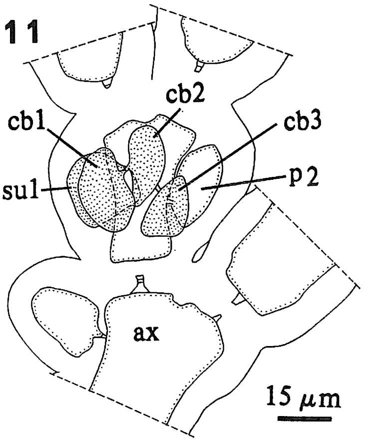

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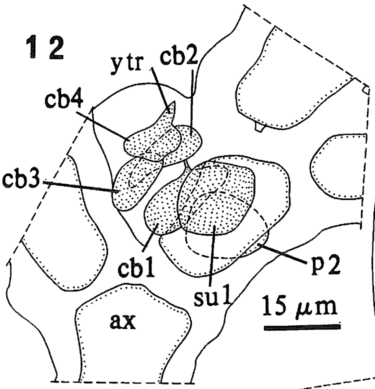

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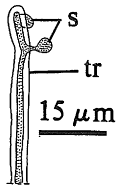

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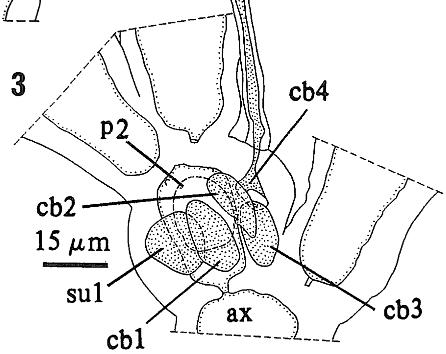

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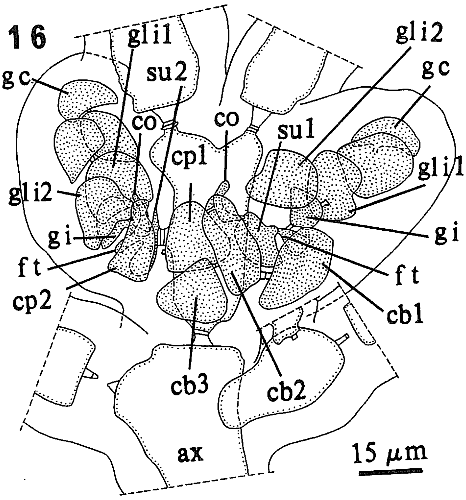

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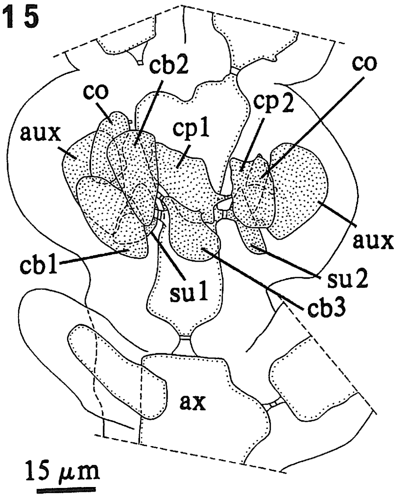

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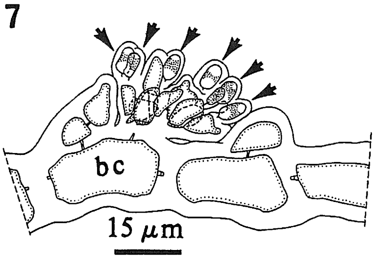

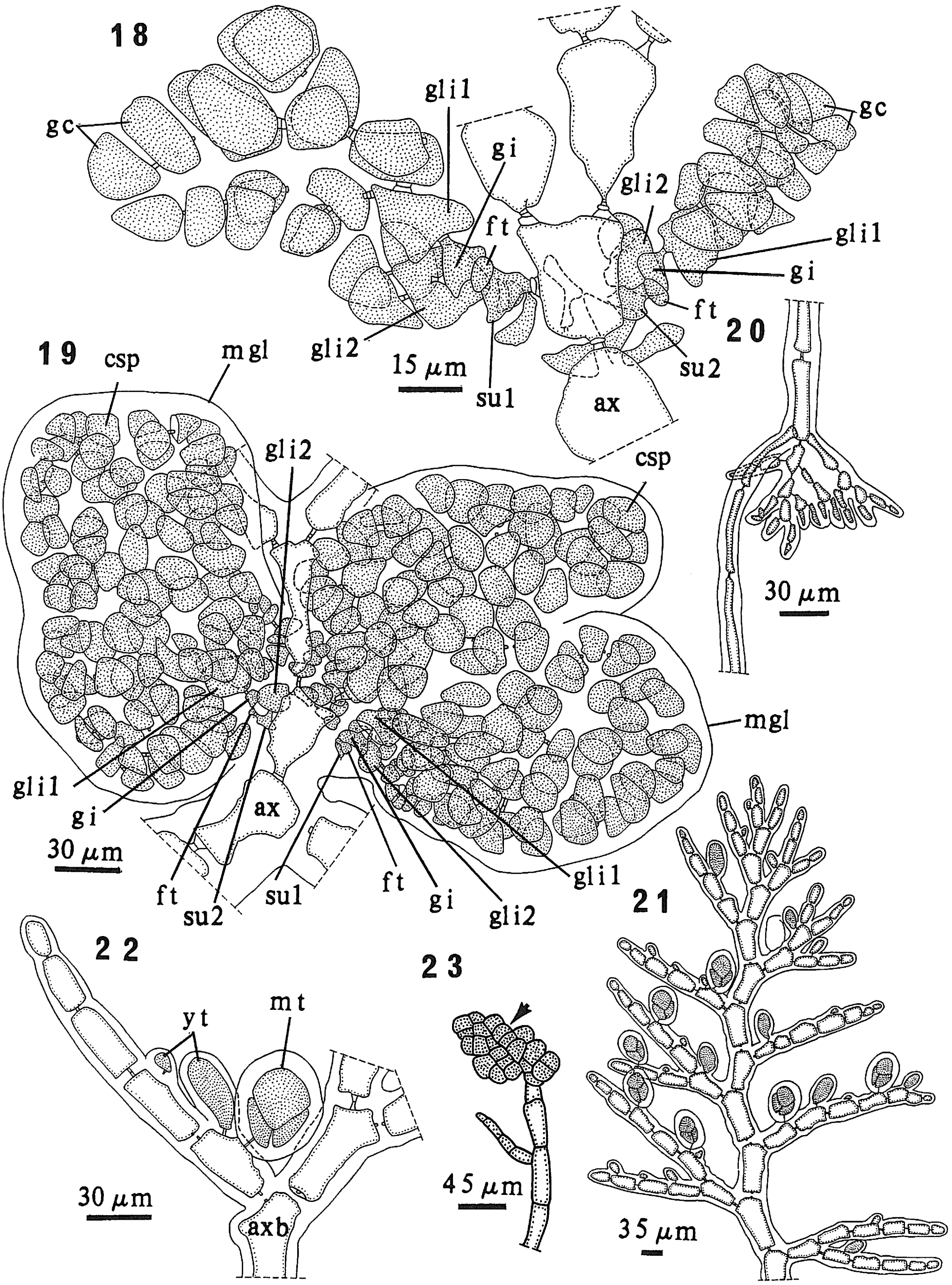
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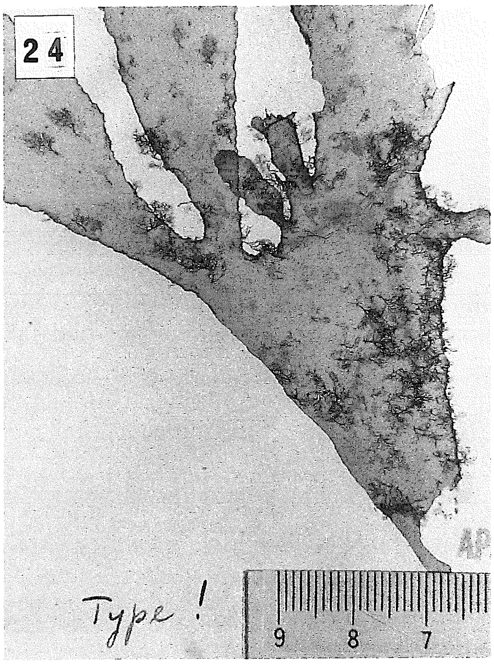

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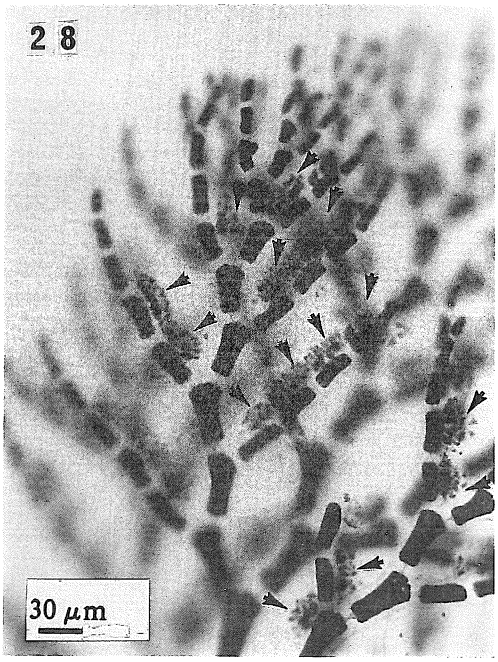

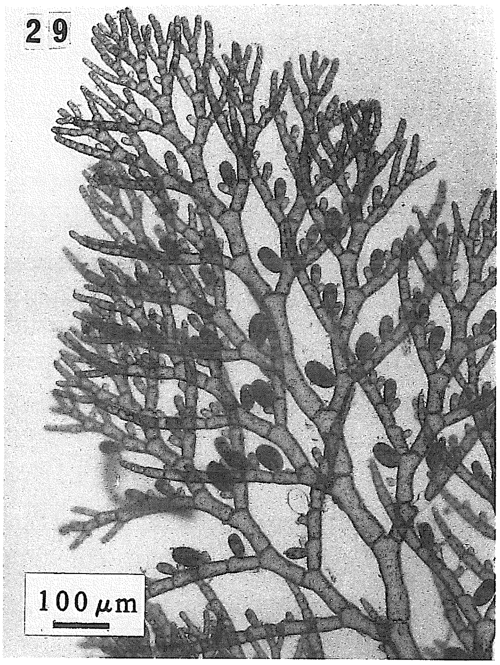

Discussion

The specimens epiphytic on *Callophyllis crispata* Okamura, collected at Enoshima by Yamada on 6 April, 1932 and mounted on a herbarium sheet, consisted of three groups (Figs. 24–26). One of the three groups of specimens was indicated "Type!" (Fig. 24) in his hand and it was reproduced photographically as pl. V in his paper but he did not use the word "type" (Yamada 1932), so it is considered properly as holotype and the other two groups labelled "Cotype!" figures 25 and 26 in his hand are considered as isotype according to Article 9.1 of the International Code of Botanical Nomenclature (Greuter *et al.* 1988).

The beach cave at Enoshima which was probably the type locality of *C. callophyllidicola* has been destroyed by a recent earthquake. However, many mature specimens of this alga were collected by the present writer in shaded places in shallow water as well as in deep water in the Oki Islands, Shimane Prefecture, in the Sea of Japan. The Oki specimens were found to be identical with the holotype in morphological features. Fertile plants of both sexes as well as tetrasporophytes were observed in the Oki Islands throughout the year.

Segawa (1942) mentioned that his material had spindle-shaped gonimolobes and some carposporophytes were provided with an involucre-like 2-3-celled short filament arising upward from the lower next axial cell to the female fertile segment. However, young gonimolobes are spindle-shaped or pear-shaped but the mature are flattened and more or less lobed and cordate, and no such involucre-like filament was detected in the present material, therefore, gonimolobes observed by Segawa (1942) seem to still be at young stage of the development. Later on, Kawashima (1960) stated, his Japanese descriptions are all translated word for word, "Each of carpogonium and the first formed connecting cell cut off the second connecting cell (co2) opposite (outward) each after development of the auxiliary cells (fig. IV, 3) (Sometimes, the second connecting cell cut off from the carpogonium further produces the third connecting cell (co3) (fig. IV, 4)) and each of those connecting cells connects with the auxiliary cell (a1, a2) which are positioned on the right and the left." However, in the present study, following presumable fertilization, the supporting cell and the second fertile pericentral cell cut off an auxiliary cell opposite the carpogonial branch on the former and distally on the latter; The carpogonium cuts off the secondary carpogonium by longitudinal division, and then each of the carpogonia produces a connecting cell, and Kawashima seems to have misinterpreted secondary carpogonium for the first formed connecting cell in his fig. IV, 3.

Kawashima (1960) again stated "Later on, auxiliary cell cuts off a gonimoblast initial which produces gonimoblast filaments sequentially. Each cell of the gonimoblast becomes carposporangium and form a cystocarp." However, in the present study, following the fusion with a connecting cell, cut off from each of the two carpogonia, each of the two auxiliary cells divides into a proximal foot cell and a distal gonimoblast initial which produces usually two gonimolobe initials in sequence and gonimolobes mature also in sequence.

Kawashima (1960) also stated "Two or three female fertile segments are successively formed (rarely singly formed)". However, in the present study, female fertile segments are always singly formed.

Kawashima (1960) again stated "The cystocarp consists of ca. 4 gonimolobes, and mature gonimolobes are terminally pointed and pear-shaped or irregularly spherical in shape." He illustrated two mature gonimolobes of ca. 75×125 μm in size. However, in the present study, mature gonimolobes are compressed, more or less lobed, cordate and 133–266×93–333 μm.

The present material is also different from that used for Kawashima's study in having some other characters such as adventitious rhizoids, terminal multicellular disc of the basal rhizoid, irregularly band-shaped chromatophores, partial heterotrichous tendency, parasporangia and no longitudinal bifission of axial cells at forks. The material used for Kawashima's study was unfortunately not available for this study. However, those various morphological differences between Kawashima's and the present materials mentioned above have led the present writer to suppose that Kawashima's material may be a distinct species.

Callithamnion callophyllidicola is closely related to *C. tripinnatum* C. Agardh (Feldmann-Mazoyer 1940 as *Aglaothamnion tripinnatum*) not only in having whorl-branches bearing 1–3 orders of branchlets expanding in the same plane as the thallus but also in four other characters such as having uninucleate vegetative cells, tetrasporangia, size of terminal cells of whorl-branches and in the size of axial cells of indeterminate branches. However, *C. callophyllidicola* is different from *C. tripinnatum* in those five characters such as branchlets on the basal axial cell of whorl-branches, arrangement of carpogonial branch cells, length of mature trichogyne, size of mature gonimolobe and producing parasporangia (Table II). *Callithamnion callophyllidicola* is also closely related to *C. decompositum* J. Agardh (Halos 1965a as *Aglaothamnion decompositum*) not only in having whorl-branches bearing 1–3 orders of branchlets which expand in the same plane as the thallus but also in having uninucleate vegetative cells and parasporangia. However, the former is different from the latter in seven characters such as branchlet on the basal axial cell of whorl-branches, the arrangement of carpogonial branch cells, the length of mature trichogyne, the size of mature gonimolobe, tetrasporangia, the size of terminal cells of whorl-branches and size of axial cells of indeterminate branches (Table II).

Callithamnion callophyllidicola is also related to another Japanese species *C. oosumiense* (Itono) comb. nov. (Basionym=*Aglaothamnion oosumiense* Itono 1971: 221, figs. 4 A-E) in having whorl-branches bearing 1–3 orders of branchlets expanding in the same plane as the thallus. However, *C. callophyllidicola* is evidently different from *C. oosumiense* in the arrangement of carpogonial branch cells (Table II), namely it is zigzag fashion in the former and U-shaped or L-shaped fashion in the latter.

The plants examined in this study are distinctively smaller than the Korean plants (Boo *et al.* 1989) in two characters, as the height of thallus and the length of mature gonimolobes.

Table II. Comparison of *Callithamnion callophyllidicola*

Character	*C. callophyllidicola*[1]	*C. tripinnatum*[2]
Branchlet on the basal axial cell of each whorl-branch	Arising adaxially and simple	Arising adaxially and ramified
Arrangement of carpogonial branch cells	In zigzag fashion	In U-shaped fashion
Length of mature trichogyne	Ca. 150 μm	Ca. 500 μm
Size of mature gonimolobe	133–266×93–333 μm	80–100×135–160 μm
Cortex	Absent	Absent
Parasporangia	Present on tetrasporophyte	Absent
Tetrasporangia	Sessile, 23–50×17–66 μm	Sessile, 50–65 μm
Size of terminal cells of whorl-branch	7×14–28 μm	8×12–20 μm
Size of axial cells of indeterminate branch	21–77×21–210 μm	30–100×35–200 μm

[1] Based on Yamada (1932), Segawa (1942, 1949) and present study; [2] based on Feldmann-Mazoyer (1940) and Halos (1965a) as *Aglaothamnion tripinnatum*; [3] based on Halos (1965b as *Aglaothamnion decompositum*); [4] based on Itono (1971 as *Aglaothamnion oosumiense*)

Acknowledgments

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and its allied apecies.

C. decompositum[3]	*C. oosumiense*[4]
Arising adaxially and ramified	Arising adaxially and simple
In U-shaped fashion	In U-shaped fashion or L-shaped fashion
Ca. 85 μm	Ca. 10 μm
50×70–90 μm	Ca. 150×180 μm
Present	Absent
Present on male gametophyte and tetrasporophyte	Absent
Sessile or pedicellate, 45×65 μm	27×70 μm
12×25–35 μm	12–18×48–90 μm
70–200×130–500 μm	27×625 μm

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Abbreviations used in Figures

a	apical cell
ax	axial cell
axb	axial cell of whorl-branch
b	basal cell of whorl-branch
bbl	basal cell of branchlet
bc	branchlet cell
bmax	basal cell of main axis
cb1, cb2, cb3, cb4	cells of the carpogonial branch (cb4=carpogonium)
co	connecting cell
cp1, cp2	primary carpogonium, secondary carpogonium

csp	carposporangium
ft	foot cell
gc	gonimoblast cell
gi	gonimoblast initial
gli1, gli2	first gonimolobe initial, second gonimolobe initial
h	hair
mgl	mature gonimolobe
mt	mature tetrasporangium
n	nucleus
p1, p2	first fertile pericentral cell, second fertile pericentral cell
rh	basal rhizoid
s	spermatium
sc	second axial cell from the base of whorl-branch
su1, su2	first supporting cell, second supporting cell
tr	trichogyne
yt	young tetrasporangium
ytr	young trichogyne