Survey of Pyrenoid Distribution in Brown Algae*

by Terumitsu HORI**

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Abstract

A study of pyrenoid distribution in field material of 52 species of brown algae was conducted with an electron microscope. Of the representatives examined, 17 species possessed pyrenoids, while the rest lacked pyrenoids.

It is interesting to note that most of the species possessing pyrenoids fall within the group of either isogamy or anisogamy, while those with lack pyrenoids fall within the group of oögamy.

Classification of the Phaeophyceae has been primarily based on the method of thallus formation and reproduction and the type of life history¹⁻⁴⁾. According to Evans⁵⁾, the first person who tried to introduce the distribution of pyrenoid in the taxonomy of the Phaeophyceae as a diagnostic character was Simon⁶⁾. However, her discussion was restricted to the results from light microscope observations. As far as I know, Evans^{5,7)} was the first to bring the results of electron microscope studies with pyrenoids in the taxonomy of the Phaeophyceae. He considered that distribution of pyrenoids was useful as an additional character in determining their taxonomic relationships. Recently, Cole and her co-workers have extensively carried out electron microscope studies of brown algae in relation to their phylogeny⁸⁻¹²⁾. They were of the opinion that the following characters could be useful as criteria for their taxonomy as well as for their classification: the position of Golgi bodies, the presence or absence of pyrenoids, type of pyrenoid, arrangement of the plasmodesmata, cell wall structure, banding pattern of chloroplast thylakoids, presence or absence of chloroplast out-pocket and others^{9,10)}.

The present study was undertaken for the purpose of providing an additional information as to the presence or absence of pyrenoids for field material of the Phaeophyceae. Studies on the fine structure of the various life history stages are now in progress, and the results will be presented in another paper.

Materials and Methods

The algae surveyed in the present study are listed in Table 1 with their species names and localities and dates of collection. The systematic arrangement presented in the list follows essentially that of Kylin¹³⁾.

The materials examined were all from vegetative portions of the specimens collected in nature.

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** Biological Institute, Faculty of Science, Toho University, Narashino, Chiba, Japan.

Table 1. Details of representatives investigated.

Species	Source	Collection
<i>Giffordia indica</i>	Nashiro, Okinawa.	Mar. 1969.
<i>Ectocarpus breviararticulatus</i>	Nashiro, Okinawa.	Mar. 1969.
<i>Botrytella micromora</i>	Muroran, Hokkaido.	Jun. 1969.
<i>Ralfsia verrucosa</i>	Augoran, Hokkaido.	Aug. 1968.
<i>Sphacelaria</i> sp.	Nabeta, Izu.	Feb. 1969.
<i>Sphacelaria</i> sp.	Muroran, Hokkaido.	Aug. 1968.
<i>Cutleria cylindrica</i>	Shichirigahama, Kanagawa.	Feb. 1968.
<i>Dictyota dichotoma</i>	Hatsuse, Kanagawa.	Feb. 1968.
<i>D. dilatata</i>	Shichirigahama, Kanagawa.	Apr. 1967.
<i>Padina arborescens</i>	Nabeta, Izu.	Feb. 1969.
<i>P. crassa</i>	Zushi, Kanagawa.	Jun. 1967.
<i>Dictyopteris undulata</i>	Nabeta, Izu.	Mar. 1968.
<i>D. divaricata</i>	Muroran, Hokkaido.	May 1968.
<i>D. prolifera</i>	Shirahama, Izu.	May 1967.
<i>Pachydictyon coriaceum</i>	Shichirigahama, Kanagawa.	Mar. 1968.
<i>Sphatoglossum pacificum</i>	Shirahama, Izu.	May 1967.
<i>Halothrix ambigua</i>	Muroran, Hokkaido.	May 1968.
<i>Petrospongium rugosum</i>	Kominato, Chiba.	Jan. 1969.
<i>Leathesia difformis</i>	Muroran, Hokkaido.	May 1968.
<i>Papenfussiella kuromo</i>	Hatsuse, Kanagawa.	Apr. 1968.
<i>Heterochordaria abietina</i>	Muroran, Hokkaido.	May 1968.
<i>Scytosiphon lomentaria</i>	Muroran, Hokkaido.	Mar. 1967.
<i>Colpomenia sinuosa</i>	Nabeta, Izu.	May 1965.
<i>C. bullosa</i>	Muroran, Hokkaido.	May 1967.
<i>Petalonia fascia</i>	Shichirigahama, Kanagawa.	Mar. 1968.
<i>Hydroclathrus clathratus</i>	Susaki, Izu.	Feb. 1968.
<i>Coilodesme japonica</i>	Muroran, Hokkaido.	Mar. 1967.
<i>Myelophycus simplex</i>	Chōjagasaki, Kanagawa.	Apr. 1967.
<i>Ishige okamurai</i>	Shichirigahama, Kanagawa.	Mar. 1968.
<i>I. sinicola</i>	Shichirigahama, Kanagawa.	Mar. 1968.
<i>Sporochnus scoparius</i>	Shirahama, Izu.	May 1967.
<i>Desmarestia viridis</i>	Muroran, Hokkaido.	Aug. 1968.
<i>D. ligurata</i>	Muroran, Hokkaido.	Aug. 1968.
<i>D. tabacoides</i>	Susaki, Izu.	May 1967.
<i>Laminaria japonica</i>	Muroran, Hokkaido.	Aug. 1968.
<i>L. religiosa</i>	Muroran, Hokkaido.	Aug. 1968.
<i>L. angustata</i>	Muroran, Hokkaido.	Aug. 1968.
<i>Kjellaniella gyrata</i>	Muroran, Hokkaido.	Aug. 1968.
<i>Agarum cribrosum</i>	Muroran, Hokkaido.	Aug. 1968.
<i>Costaria costata</i>	Muroran, Hokkaido.	Aug. 1968.
<i>Eisenia bicyclis</i>	Muroran, Hokkaido.	Aug. 1968.
<i>Ecklonia cava</i>	Susaki, Izu.	May 1967.
<i>Eckloniopsis radicata</i>	Susaki, Izu.	May 1967.
<i>Undaria pinnatifida</i>	Shichirigahama, Kanagawa.	Feb. 1969.
<i>Alaria crassifolia</i>	Muroran, Hokkaido.	May 1968.
<i>Fucus evanescens</i>	Muroran, Hokkaido.	May 1968.
<i>Sargassum thunbergii</i>	Susaki, Izu.	May 1967.
<i>S. nigrifolium</i>	Susaki, Izu.	May 1967.
<i>S. confusum</i>	Muroran, Hokkaido.	May 1968.
<i>S. sagamianum</i>	Susaki, Izu.	May 1967.
<i>S. ringgoldianum</i>	Susaki, Izu.	May 1967.
<i>Hizikia fusiforme</i>	Nabeta, Izu.	Mar. 1968.

Materials were fixed in glutaraldehyde at 4-6° (3% or 6% in phosphate buffer pH 7.0, or 6% in sea water) and post-fixed in OsO_4 at 4-6° (1% or 2% in phosphate buffer pH 7.0) or in unbuffered KMnO_4 (2%) at room temperature. Part of each material was also fixed only in OsO_4 (2% in phosphate buffer pH 7.0). After rinsed for 15 minutes with several changes of buffer, they were dehydrated in a graded series of ethyl alcohol and propylene oxide and were embedded in Epon 812¹³⁾. Sections were cut with glass knives on a Porter-Blum microtome and stained in lead citrate¹⁴⁾, and examined with a JEM-7 electron microscope.

Observations

Ectocarpales: Four representatives of this order were examined in the present study. The occurrence of pyrenoids was confirmed in the chloroplasts of three species of the *Ectocarpus*-complex; *Ectocarpus breviarticulatus*, *Giffordia indica*, and *Botrytella micromora* (Fig. 1): but no pyrenoid was detected in *Ralfsia verrucosa** (Fig. 2). Regarding the *Ectocarpus*-complex, these results are in good agreement with those of previous works by Gibbs¹⁵⁾, Giraud¹⁶⁾, Bouck¹⁷⁾, and Evans⁵⁾. Regarding the pyrenoid in *Ralfsia*, however, the present result is in contradiction to that of Loiseaux¹⁸⁾ who reported the presence of pyrenoids in *Ralfsia clavata*. Since the heterogeneity of this genus has been the subject of recent culture studies, this contradiction suggests the need for further investigation of the fine structure in connection with the life history.

Sphacelariales: Two species of *Sphacelaria* were examined in this study and no pyrenoid was detected in their chloroplasts. This result agrees with that of Simon⁶⁾. However, according to Evans⁵⁾ the zoospores of *S. bipinnata* possess pyrenoids. Accordingly, his result also suggests the need for further observations on different stages in the life history of this genus.

Cutleriales: In *Cutleria cylindrica*, pyrenoids were detected only in a few chloroplasts of the cortical cells. These pyrenoids are all extremely small, measuring less than 1.0μ in length as well as in width. They seem to be very poorly developed (Fig. 3). They are always located at the edge of the chloroplasts, protruding into the cytoplasm. All of the vegetative cells except those of cortical portion seem to lack pyrenoids. According to previous works of Simon⁶⁾ and Evans⁵⁾, however, no alga in this genus possessed pyrenoids. Detailed discussions are not possible at the present time because these authors mentioned neither the species names nor the portion of thallus they used.

Dictyotales: Nine representatives of this order were examined, but no evidence of the occurrence of pyrenoids was detected in their chloroplasts (Fig. 4). This result corresponds well to that of Ueda¹⁹⁾, Berkalo²⁰⁾ and Evans⁵⁾. In a recent paper, Liddle and Neushal²¹⁾ reported the presence of pyrenoids in *Zonaria farlowii* in vegetative cells as well as in egg cells. However, it is rather strange to me that there is no structure corresponding to pyrenoid in their text photomicrographs.

* Identification of this alga was kindly made by Professor Y. Nakamura, Algological Research Institute, Faculty of Science, Hokkaido University, to whom I am indebted.

Chordariales: Eight species of this order were examined in this study and pyrenoids were found in seven representatives (Figs. 5, 10, 12) (cf. Table 2). As far as I know, three species were previously studied with an electron microscope and all have been known to possess pyrenoids: *Myrionema orbiculare* by Loiseaux¹⁸⁾, *Leathesia difformis* by Cole and Lin¹⁰⁾ and *Eudesme virescens* by Cole¹¹⁾.

In connection with the present results, a few comments should be mentioned regarding the systematic position of *Heterochordaria abietina*. As to this species, Nakamura²²⁾ discussed its similarity to *Ralfsia verrucosa*, a species of *Ralfsia sensu stricto*, on the basis of the method of thallus formation and the type of life history. He is of the opinion that these two algae should be placed in the same taxon. As described above, my study confirmed that these two algae have no pyrenoids. This evidence seems to provide an additional criterion to Nakamura's classification.

In the present study I have not been able to find pyrenoids in the chloroplast of *Ishige sinicola*. The question arises at once whether it lacks pyrenoids completely or it may rarely possess pyrenoids, since in a related alga, *I. okamurai* (Fig. 12), pyrenoids were very rarely recognized in this investigation. Further study to confirm it is now under way.

Punctariales: Six representative were examined (Table 1) and all of them possess pyrenoids (cf. Table 2). Their pyrenoids are fairly large, and hence they are easily recognized (Figs. 6-9).

Sporochneales and *Desmarestiales*: No pyrenoid was detected in such representatives of these orders as *Sporochnus scoparius*, *Desmarestia tabacoides* (Fig. 11), *D. ligulata* and *D. viridis*.

Laminariales: The following eight species of this order were examined—*Kjellmaniella gyrata*, *Agarum cribrosum*, *Costaria costata*, *Eisenia bicyclis*, *Ecklonia cava*, *Eckloniopsis radicata*, *Undaria pinnatifida* (Fig. 13), and *Alaria crassifolia*. No pyrenoid could be identified in all of them except *Undaria pinnatifida* in which a few chloroplasts possess a small lateral bud-like body that protrudes into the cytoplasm (Fig. 13). At present, I cannot say definitely whether it is a sort of "rudimentary pyrenoid"⁷⁾ or "out-pocket"⁹⁻¹¹⁾. The results concerning other species agree with the findings of Evans⁶⁾ and Simon⁶⁾.

Fucales: Seven species of three genera were investigated—*Sargassum ringgoldianum* (Fig. 15), *S. nigrifolium* (Fig. 16), *S. thunbergii*, *S. confusum*, *S. sagamanum*, *Hizikia fusiforme* (Fig. 14), and *Fucus evanescens*, but no pyrenoid was encountered. According to Evans⁷⁾, a vestigial pyrenoid was present in the egg chloroplasts of British fucoids, *Bifurcaria bifurcata*, *Himanthalia elongata* and *Cystoseira tamariscifolia*, but not in those of *Ascophyllum nodosum*, *Fucus serratus*, *F. spiralis*, *Pelvetia canaliculata* and *Halidrys siliquosa*, or in the vegetative cells of *B. bifurcata* and *C. tamariscifolia*.

Discussion

All species of brown algae that have been examined so far with an electron microscope are listed in Table 2, which includes about eighty species of fifty-one genera. From the results of the present study and the data available at the present

Fig. 1. *Botrytella micromora*. A chloroplast with a pyrenoid. ($\times 9,800$)

Fig. 2. *Ralfsia verrucosa*. A chloroplast without a pyrenoid. ($\times 11,000$).

Fig. 3. *Cutleria cylindrica*. A part of a chloroplast with a pyrenoid. ($\times 61,000$).

Fig. 4. *Pachydictyon coriaceum*. A chloroplast showing the lack of a pyrenoid ($\times 23,000$).

Key to abbreviations. m: mitochondria; N: nucleus; p: pyrenoid; w: cell wall.

Fig. 5. *Petrospongium rugosum*. A chloroplast with a pyrenoid in thallus cell. ($\times 9,400$).

Fig. 6. *Scytosiphon lomentaria*. A chloroplast and non-median section of pyrenoid. Tubule (arrow) extending from nucleus in visible. ($\times 11,900$).

Fig. 7. *Colpomenia bulbosa*. Section of a chloroplast with a pyrenoid. Also visible are a pair of membranes extending from the outer nuclear membrane (arrow). ($\times 16,000$).

Fig. 8. *Coilodesme japonica*. A chloroplast with two stalked pyrenoids near both ends. ($\times 12,500$).

Fig. 9. *Hydroclathrus clathratus*. A chloroplast with a pyrenoid. ($\times 8,700$).

Fig. 10. *Myelophycus simplex*. A part of a chloroplast with an unusual shape of pyrenoid. ($\times 16,300$).

Fig. 11. *Desmarestia tabacoides*. Many chloroplasts without pyrenoids. A cell is enclosed about half way around by some separate thin layers of cell wall. ($\times 2,800$).

Fig. 12. *Ishige okamurai*. A chloroplast bearing a small pyrenoid. ($\times 24,400$).

Fig. 13. *Undaria pinnatifida*. A chloroplast bearing the bud-like body resembling a rudimentary pyrenoid. ($\times 19,900$).
 Fig. 14. *Hizikia fusiforme*. A chloroplast without a pyrenoid. ($\times 22,300$).
 Fig. 15. *Sargassum ringgoldianum*. A chloroplast without a pyrenoid. ($\times 27,000$).
 Fig. 16. *S. nigrifolium*. Chloroplasts without a pyrenoid. ($\times 11,900$).

Table 2. List of brown algae investigated with an electron microscope.

Species	Presence or absence of pyrenoid ¹		References
	in reproductive cells ²	in vegetative cells ²	
Ectocarpales:			
<i>Ectocarpus breviarticulatus</i>		+	
<i>E. confervoides</i>	(+)		5.
<i>Giffordia indica</i>		+	
<i>Giffordia</i> sp.		(+)	17.
<i>Pilayella</i> sp.		(+) ⁴	16.
<i>Pilayella littoralis</i>	(+)	(+)	5, 15.
<i>Botrytella micromora</i>		+	
<i>Ralfsia verrucosa</i>		—	
<i>R. clavata</i>		(+) ⁴	18.
Sphacelariales:			
<i>Sphacelaria bipinnata</i>	(+)		5.
<i>Sphacelaria</i> sp.		—	
<i>Sphacelaria</i> sp.		—	
Cutleriales:			
unknown species ³		(—) ⁴	5.
<i>Cutleria cylindrica</i>		+	
Dictyotales:			
<i>Dictyopteris membranacea</i>		(—) ⁴	5.
<i>D. divaricata</i>		—	
<i>D. prolifera</i>		—	
<i>D. undulata</i>		—	
<i>Dictyota dichotoma</i>	(—)	(—), —	5, 23.
<i>D. dilatata</i>		—	
<i>Pachydictyon coriaceum</i>		—	
<i>Padina arborescens</i>		(—) ⁴	19.
<i>P. crassa</i>		—	
<i>P. pavonia</i>		(—)	5.
<i>Sphatoglossum pacificum</i>		—	
Chordariales:			
<i>Halothrix ambigua</i>		+	
<i>Leathesia difformis</i>		(+), +	10.
<i>Petrospongium rugosum</i>		+	
<i>Papenfussiella kuromo</i>		+	
<i>Heterochordaria abietina</i>		—	
<i>Myelophycus simplex</i>		+	
<i>Ishige okamurai</i>		+	
<i>I. sinicola</i>		—	
<i>Myrionema orbiculare</i>		(+) ⁴	18.
<i>Eudesme virescens</i>		(+)	11.

(continued)

Punctariales:

<i>Colpomenia bullosa</i>		+	
<i>C. peregrina</i>	(+)		5.
<i>C. sinuosa</i>		+	
<i>Hydroclathrus clathratus</i>		+	
<i>Petalonia fascia</i>		+	
<i>Phaeostrophion irregulare</i> ⁵		(+, -)	9.
<i>Scytosiphon lomentaria</i>	(+), +	(+), +	5, 25.
<i>Coilodesme japonica</i>		+	

Dictyosiphonales:

<i>Dictyosiphon foeniculaceus</i>		(+)	5.
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Sporochneales:

unknown species ³		(-) ⁴	5.
<i>Sporochnus scoparius</i>		-	

Desmarestiales:

<i>Desmarestia ligulata</i>		-	
<i>D. viridis</i>		-	
<i>D. tabacoides</i>		-	

Laminariales:

<i>Chorda filum</i>	(-)	(+)	5, 17.
<i>Agarum cribrorum</i>		-	
<i>Costaria costata</i>		-	
<i>Kjellmaniella gyrata</i>		-	
<i>Laminaria angustata</i>		-	
<i>L. digitata</i>	(-)		5.
<i>L. japonica</i>		-	
<i>L. religiosa</i>		-	
<i>Saccorhiza polyschides</i>	(-)		5.
<i>Alaria crassifolia</i>		-	
<i>A. esculenta</i>	(-)		5.
<i>Ecklonia cava</i>		-	
<i>Eckloniopsis radicata</i>		-	
<i>Egregia menziesii</i>		(-)	24.
<i>Eisenia bicyclis</i>		-	
<i>Undaria pinnatifida</i>		+?	

Fuciales:

<i>Ascophyllum nodosum</i>	(-)		8.
<i>Fucus evanescens</i>		-	
<i>F. serratus</i>	(-)		8.
<i>F. spiralis</i>	(-)		8.
<i>F. vesiculosus</i> ⁶		(-)	17, 26.
<i>Pelvetia canaliculata</i>	(-)		8.
<i>Himanthalia elongata</i>	(+)		8.
<i>H. lorea</i>		(-)	20.
<i>Bifurcaria bifurcata</i>	(+)	(-)	8.

(continued)

<i>Cystoseira tamariscifolia</i>	(+)	(-)	8.
<i>Halidrys siliquosa</i>	(-)		8.
<i>Sargassum confusum</i>		-	
<i>S. nigrifolium</i>		-	
<i>S. ringgoldianum</i>		-	
<i>S. sagamianum</i>		-	
<i>S. thunbergii</i>		(-), -	19.
<i>Hizikia fusiforme</i>		-	

¹ In this table a mark of plus shows the presence of pyrenoids, and a mark of minus indicates that the pyrenoid has not yet been observed.

² In this rank, egg, zoospore and settled spore were involved.

³ In original the species name was not described.

⁴ Parentheses in two ranks, e.g., reproductive and vegetative cells, indicate the results being studied by previous observers, but when placed in the middle they indicate that the part of thallus was not mentioned.

⁵ This alga was described as a member of the Dictyosiphonales in original⁹.

⁶ This alga has been observed by many electron microscopists.

time, the following conclusions may be drawn.

First, it is interesting to note that great majority of algae which contain pyrenoids are the members belonging to more primitive taxa. These members are of the algae of either isogamy or anisogamy in the method of their reproduction. However, there are a few exceptions such as *Ralfsia verrucosa* (Ectocarpales), *Heterochordaria abietina* (Chordariales), and possibly *Ishige sinicola* (Chordariales).

Second, the members of the Dictyotales, Laminariales, Sporochneales, and Desmarestiales lack pyrenoids. It should be noted that these members are all of the algae of oögamy in the method of reproduction.

Third problem concerns the pyrenoids of *Cutleria cylindrica*. To judge from the photographs given by Evans⁷ of the rudimentary type of pyrenoid in fucalean egg chloroplasts, such a pyrenoid seems to fall within the range from 0.5 to 1.0 μ in size, in width as well as in length. The pyrenoid of *C. cylindrica* is also very small and measures about 0.5 μ in width and about 0.9 μ in length, a size well in correspondence with the so-called rudimentary pyrenoid of Evans. The usual type of pyrenoid in most of brown algae is in the range from 1.5 to 3.0 μ in width and from 1.5 to 2.5 μ in length (Hori, unpublished data). These evidences appear to suggest that there is a gradation in characteristics from algae bearing a usual type of pyrenoid and having either isogamous or anisogamous reproduction, through algae with a rudimentary type of pyrenoid and with either anisogamous or oögamous reproduction, to those bearing no pyrenoid and having oögamous reproduction. If we accept this hypothesis, the present study could support the view of Kylin¹ that the Cutleriales in a taxon to be placed systematically between the Ectocarpales and the Tilopteridales-Dictyotales.

Fourth, as discussed briefly in the preceeding pages, in some cases pyrenoid distribution is not in agreement with each other between vegetative cells and reproductive ones even in identical species or the closely related taxa. As far as the present study is concerned, species of *Sphacelaria* and those of the Fucales show such an example. As has been emphasized by Cole and her co-workers^{9,11}, it should surely be important to study fine structures of algae in various stages of

their life history.

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Addendum

After the present manuscript was completed, one paper by Dr. Cole on the ultrastructural study of three species in the order Scytosiphonales appeared in *Phycologia*, volume 9(3/4), 1970. According to her paper, the presence of pyrenoids was detected in the chloroplasts of field material of *Colpomenia sinuosa*, *Petalonia debilis* and *Scytosiphon lomentaria*, and of sporeling of *Petalonia*. Her findings regarding the field material are in accord with mine.

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