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The generic delimitation of *Rhodella* (Porphyridiales, Rhodophyta) with emphasis on ultrastructure and molecular phylogeny

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Abstract

We investigated the cellular features and molecular phylogeny of *Rhodella* species and related unicellular red algae including undescribed species that we isolated. Results provide a new taxonomic interpretation at both generic and specific levels. The genus *Rhodella* is defined by its pyrenoid that is free from any internal structures. Based on phylogenetic analysis using 18SrDNA, there are two possibilities for the generic delimitation of *Rhodella*: *Rhodella sensu stricto* and *Rhodella sensu lato*. The generic autonomy of *Dixoniella* and the taxonomic position of *R. cyanea* were also discussed.

Introduction

When Evans (1970) established the genus *Rhodella* with the type species *R. maculata*, he considered that the ultrastructural characteristics were sufficient to separate it from the other unicellular reds, such as *Porphyridium*, *Rhodosorus* and *Cyanidium*. He was the first to do such an ultrastructural taxonomic study on unicellular reds. According to him, the following morphological and ultrastructural characters appear in *R. maculata* (Evans 1970); (1) the chloroplast is axile and highly-lobed stellate with a naked pyrenoid; (2) the pyrenoid is free from any structures, except a tongue-shaped nuclear invagination and is covered with floridean starch shells; (3) the nucleus is not situated in the central portion of the cell. However, this initial diagnosis did not provide any clear delimitation between the generic and specific characters in *Rhodella*.

A second species was reported by Wehrmeyer (1971), who transferred *Porphyridium violaceum* Kornmann to *Rhodella* as *R. violacea*, since its ultrastructural features were basically the same as those of *R. maculata* with only one distinction; the apparent absence of a nuclear projection into the pyrenoid. However, Patrone et al. (1991) found a small nuclear projection into the pyrenoid of *R. violacea* from the type culture. It shows the necessity to re-examine the two species to determine whether they are conspecific or not.

A third species, *Rhodella reticulata* Deason, Bulter *et* Rhyne described by Deason et al. (1983), possesses a highly-lobed chloroplast with a naked pyrenoid containing convoluted thylakoids in the matrix. Fresnel et al. (1989) pointed out that this species is conspecific with *Porphyridium griseum* Geitler since the same chloroplast ultrastructure was found and made a new combination of *Rhodella grisea* (Geitler) Fresnel, Bellard, Hindák *et* Pekárková. According to Scott et al. (1992), however, the ultrastructural features of this species, such as pyrenoid structure, dictyosome arrangement, nuclear position in the cell, relative configuration of pyrenoid and nucleus, are different from those of the former two *Rhodella* species. Scott et al. (1992) consequently established the genus *Dixoniella* and placed *R. reticulata* under this genus in synonymy

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Table 1. List of *Rhodella* species and related taxa examined in this study

Species	Strain name	Collection site/Source	TEM	Accession no.
Rhodella cyanea	Tobishima	Tobishima Island, Yamagata, Japan	+	AB045605
R. maculata	Amami	Amami Island, Kagoshima, Japan	+	AB045608
R. violacea	BRW	Point Barrow, Elson Lagoon, Alaska, USA	+	AB045604
	B115.79	SAG*[1]		AB045580
Rhodella species	Nagura 1	Nagura River, Ishigaki Island Okinawa, Japan	+	AB045598
Rhodella sp.	Gamou T4	Gamou Tideland, Miyagi, Japan	+	AB045591
*R*hodella sp.	Mexico BC73C	Mulege River, Baja California Sur, Mexico	+	AB045594
Dixoniella grisea	B39.94	SAG*[1]		AB045581
	Ogasawara	Chichi Island, the Ogasawara Islands, Tokyo, Japan	+	AB045583
Glaucosphaera vacuolata	1662	UTEX*[2]		AB045583
Porphyridium purpureum	R-1	IAM*[3]		AB045584

TEM=Transmission Electron Microscopy. Plus (+): studied. *[1]SAG: Sammlung von Algenkulturen at the University of Göttingen. *[2]UTEX: The Culture Collection of Algae at the University of Texas at Austin, Department of Botany, University of Texas. *[3]IAM: Institute of Applied Microbiology, The University of Tokyo (the present name: Institute of Molecular and Cellular Biosciences (IMCB), The University of Tokyo).

with *Dixoniella grisea* Scott, Broadwater, Saunders, Thomas *et* Gabrielson.

A fourth species, *Rhodella cyanea,* was described by Billard & Fresnel (1986). The configurations of the pyrenoid, nucleus and other organelles are quite unique. However, the naked pyrenoid contains convoluted thylakoids in the matrix, like those of *D. grisea* (Scott et al., 1992). The subcellular organization, with a central nucleus, perinuclear dictyosomes and multilobed chloroplast, is similar to those of *D. grisea* and *Glaucosphaera vacuolata* Korshikov (Broadwater et al., 1995). Because of the blue-green cell color, *R. cyanea* was thought to lack phycoerythrin (Billard & Fresnel, 1986), whereas *R. maculata* and *R. violacea* contain Bangiophycean type of phycoerythrin (Koller & Wehrmeyer, 1975; Billard & Fresnel, 1986). For the reason stated above it appears incorrect to assign *R. cyanea* to *Rhodella.*

From the historical background of unicellular red algal investigations focused on *Rhodella*, the following taxonomic questions remain; How is the genus *Rhodella* defined? What is the taxonomic position of *R. cyanea*? What is the relationship among *Rhodella*, *Dixoniella* and *Glaucosphaera*?

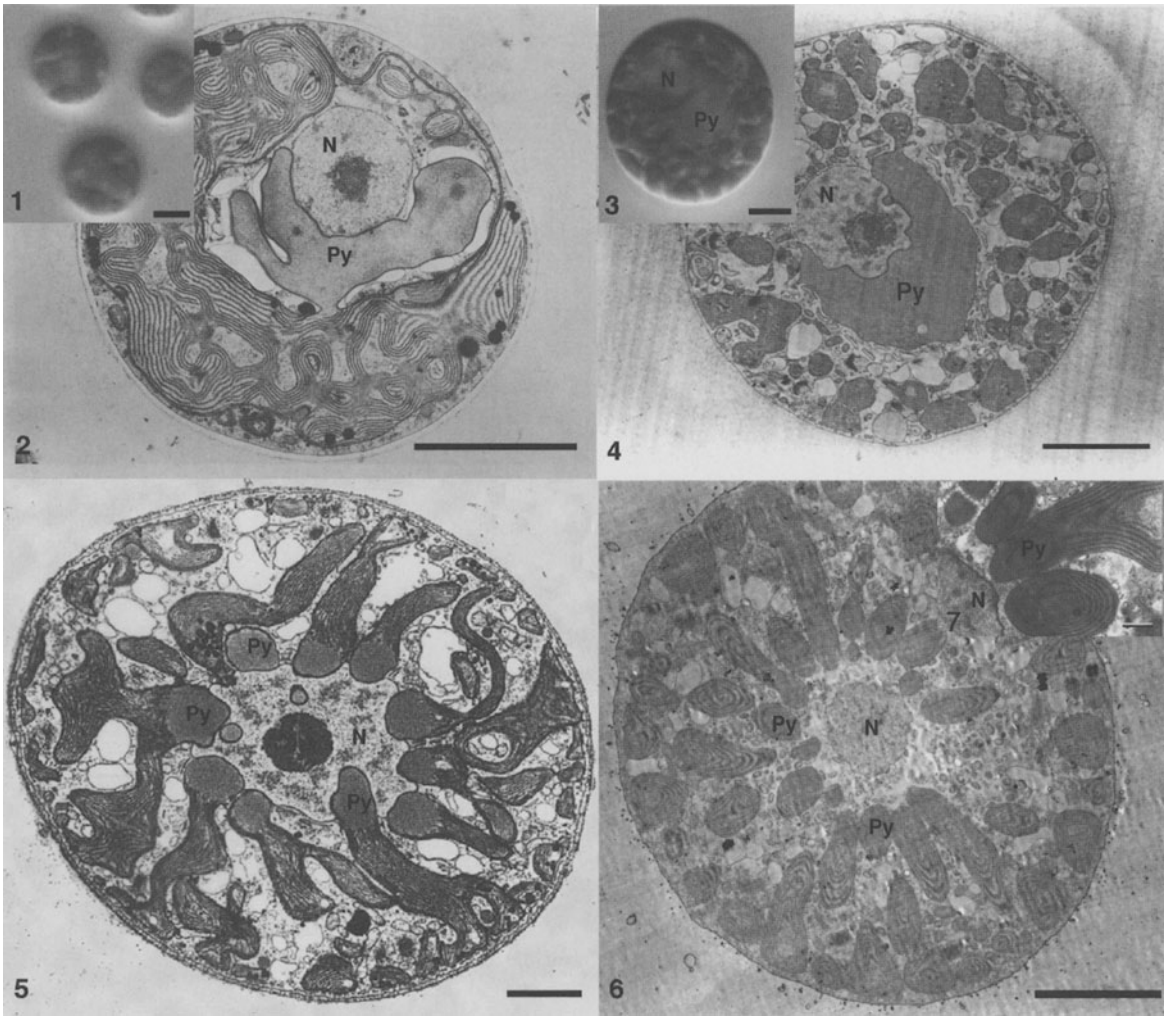
In order to elucidate these questions, we observed the cellular features of *Rhodella* including several undescribed species originally isolated by us, and determined their 18SrDNA sequences and constructed a phylogenetic tree.

Materials and methods

The Porphyridialean algae examined in this study are listed in Table 1, together with their culture names and collection sites or institutions maintaining them. All of them were grown in ESM medium (Okaichi et al., 1982) and maintained at 20 °C under 14:10 L:D cycle. Light was provided by cool white fluorescent lamps at ca. 20 μmol m^{-2} s^{-1}. Light microscope studies were made on cultured live cells, and transmission electron microscope studies were done on fixed materials by the methods published in a previous report (Hara & Chihara, 1985).

Total DNAs were extracted by modified 2xCTAB method developed by Hasebe & Iwatsuki (1990). PCR amplification was performed by the method of Hasebe et al. (1994). The genes of 18SrDNA were amplified with SR primers (Nakayama et al., 1996). The sequences were determined from the PCR products with Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems) on ABI 373A or ABI 310 sequencers. Sequences obtained in this study were aligned manually, including the published data of *Erythrotrichia carnea* L26188, *Dixoniella grisea* L26187 and *Rhodella maculata* U21217 (Ragan et al., 1994). The phylogenetic tree was constructed by fastDNAml (Olsen et al., 1994) and bootstrap values were calculated by PAUP ver. 3.1.1 (Swofford, 1993).

Phycobiliproteins were extracted and analyzed by the following procedure. Cultured cells were pelleted by centrifugation (1500 ***g***, 5 min). The pellets were suspended in 10 mM potassium phosphate buffer



Figures 1–7. Light (Figs 1 and 3) and transmission electron (Figs 2 and 4–7) micrographs of *Rhodella* species. Bars: 5 μm (Figs 1–6), 0.5 μm (Fig. 7). **Figure 1**. Cells of *Rhodella* sp. (Nagura strain) showing a highly lobed stellate chloroplast. **Figure 2**. A cell of *Rhodella* sp. (Nagura st.) showing the subcellular features, in particular, configurational relation between nucleus (N) and pyrenoid (Py). **Figure 3**. Cells of *Rhodella* sp. (Gamou st.) showing the location and shapes of a nucleus and chloroplast with pyrenoid. **Figure 4**. A cell of *Rhodella* sp. (Gamou st.) showing the subcellular features similar to those of Nagura strain. **Figure 5**. A cell of *Rhodella* sp. (Mexico st.) showing the unique configuration between a nucleus and chloroplast-pyrenoid. **Figure 6**. A cell of *R. cyanea* (Tobishima st.) showing similar subcellular features as holotype of *R. cyanea* (Billard & Fresnel, 1986). **Figure 7**. A central part of the cell of *R. cyanea* (Tobishima st.) showing thylakoids in the chloroplast entering into the pyrenoid matrix.

(pH 6.85). The cells were broken by ultra sonicator UD-200 (TOMY SEIKO Co. Ltd., Tokyo) or were vortexed with glass beads. After centrifugation (20 000 ***g***, 15 min, 4 °C), the supernatant was transferred to a new tube and saturated with ammonium sulfate. After stirring 2 h at 4 °C, the samples were dialyzed in 5 mM potassium phosphate buffer (pH 6.85). Chromatography was performed by DEAE-Cellulofine A-500 (Seikagaku Co., Tokyo) gradient from 5 mM to 150 mM potassium phosphate buffer. Phycobiliproteins of all fractions were measured by a spectrophotometer UV-200 (SHIMADZU Co., Kyoto).

Results and discussion

Previously described species of *Rhodella* (*R. maculata, R. cyanea, R. violacea*) and three undescribed species (provisionally named as *Rhodella* sp. and followed by Nagura, Gamou and Mexico strains in the

Table 2. Morphological and ultrastructural comparison of *Rhodella* and related taxa

	Cell size (μm)	Chloroplast	Pyrenoid		Nucleus		Dictyosome*[1]	Pigment	Reference
		Location	Type	Internal structures	Location	Projection into pyrenoid	Association	Phycoerythrin type	
Rhodella maculata	7–24	Axile	Naked	Absent	Peripheral	Present	ER	B	Evans (1970)
R. violacea	8–30	Axile	Naked	Absent	Peripheral	Absent[2]*	ER	B	Wehmeyer (1971)
R. sp. (Nagura st.)	7–13	Peripheral	Naked	Absent	Peripheral	Absent	ER?	B	This study
R. sp. (Gamou st.)	19–40	Peripheral	Naked	Absent	Peripheral	Absent	ER?	B	This study
R. sp. (Mexico st.)	18–35	Axile	Naked	Absent	Central	Absent	ER?	B	This study
R. cyanea (Tobishima st.)	20–28	Axile	Naked	Thylakoid	Central	Absent	Nu?	B	This study
R. cyanea	22–40	Axile	Naked	Thylakoid	Central	Absent	Nu	ND?	Billard & Fresnel (1986)
Dixoniella grisea	8.5–17	Axile	Naked	Thylakoid	Peripheral	Absent	Nu	C*[3]	Scott et al. (1992)
Glaucosphaera vacuolata	14–22	Peripheral	Absent	–	Central	Absent	Nu	ND	Broadwater et al. (1995)
Porphyridium purpureum	6–12*[4]	Axile	Embedded	Thylakoid	Peripheral	Absent	ER/M	B,b	Schornstein & Scott (1982)

*[1]Dictyosome data except for ER? and Nu? were referred to Broadwater & Scott 1994. *[2]Nuclear projection into pyrenoid was observed in *R. violacea* by Patrone et al. (1991). *[3]Phycoerythrin type of *Dixoniella* was determined in this study. *[4]Cell size of *P. purpureum* was examined in this study from *P. purpureum* R-1 strain. ER: endoplasmic reticurum, ER?: Dictyosomes are not faced to nucleus but probably to ER, Nu: Nucleus, B: Bangiophycean type of phycoerythrin, b: modified type of B-phycoerythrin, C: Cyanophyoean type of phycerythrin, ND? or ND: Not detected.

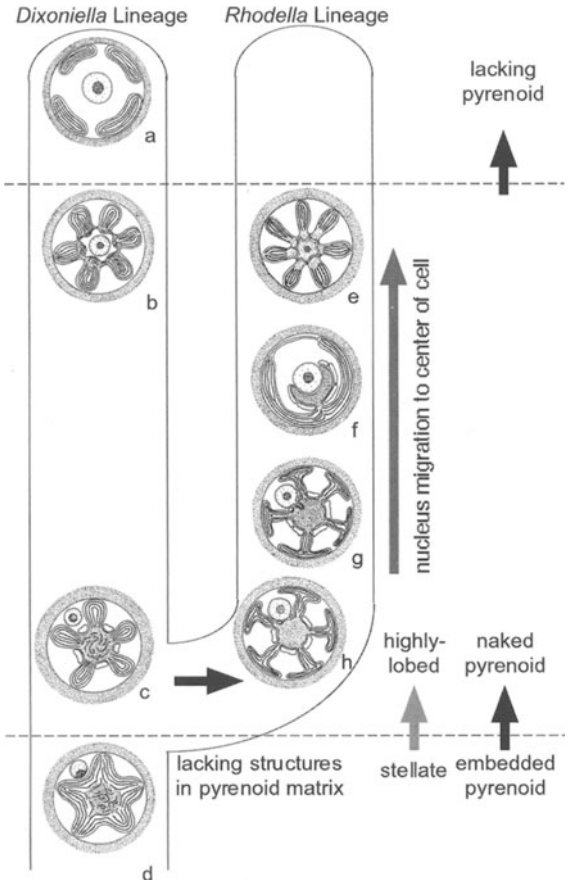


Figure 8. A hypothetical scheme of the relationship of *Rhodella* and its related species based on the structural characteristics. Arrows show the structural clines focused on chloroplast, pyrenoids and nucleus. (a) *Glaucosphaera vacuolata*, (b) *Rhodella cyanea*, (c) *Dixoniella grisea*, (d) *Porphyridium purpureum*, (e) *Rhodella* species (Mexico st.), (f) *Rhodella* sp. (Nagura st.) and *Rhodella* sp. (Gamou st.), (g) *R. maculata*, (h) *R. violacea*.

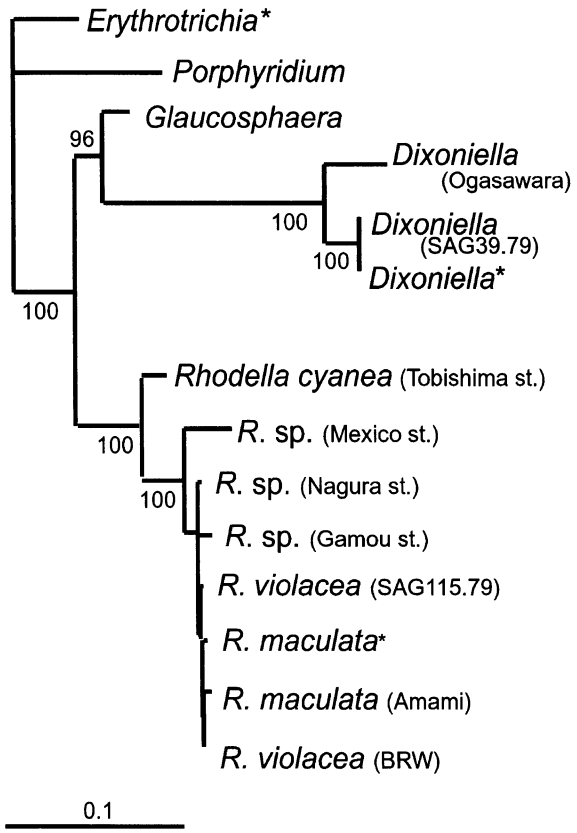


Figure 9. Maximum likelihood tree (fastDNAml) of 18SrDNA sequences, based on the conservative regions (1458 nucleotides). The numbers on branches indicate bootstrap percentages (1000 replicates, without less than 50%) which are calculated by PAUP 3.1.1 (Swofford, 1993). Three species indicated by the asterisks were sequenced by Ragan et al. (1994).

parentheses) were investigated by combining their cellular features with molecular phylogenetic analysis using 18SrDNA to provide a new interpretation of their generic and specific taxonomy.

In *Rhodella* sp. (Nagura strain), a unique configuration and structures of organelles were found (Fig. 2). The chloroplast with a naked pyrenoid is parietal and situated in the cell periphery. The amorphous pyrenoid is connected to the chloroplast by a narrow isthmus and surrounds half the nuclear surface. The pyrenoid matrix is free from any internal structures such as thylakoids and tubular structures, a ultrastructural characteristic that is basically shared with *Rhodella maculata* (Evans, 1970) and *Rhodella violacea* (Wehrmeyer, 1971).

An organellar configuration and pyrenoid ultrastructure similar to those of *Rhodella* sp. (Nagura st.) were recognized in *Rhodella* sp. (Gamou st.). The surface of the nucleus is partly enclosed by the pyrenoid and the pyrenoid matrix lacks thylakoids (Fig. 4). The cell size range (20–40 μm diam.) of *Rhodella* sp. (Gamou st.), however, is considerably larger than that (7–13 μm diam.) of *Rhodella* sp. (Nagura st.) (Figs 1 & 3).

Rhodella sp. (Mexico st.) also has a unique organellar configuration and pyrenoid structure (Fig. 5). A stellate chloroplast is highly lobed; the pyrenoid and nucleus configuration closely resembles that of *R. cyanea* (Billard & Fresnel, 1986) and the Tobishima strain examined in this study (Figs 6 and 7). However, some differences were found between *Rhodella* sp. (Mexico st.) and *R. cyanea*. The pyrenoid matrix of *Rhodella* sp. (Mexico st.) is free from any internal structures (Fig. 5), but that of *R. cyanea* is invaded by

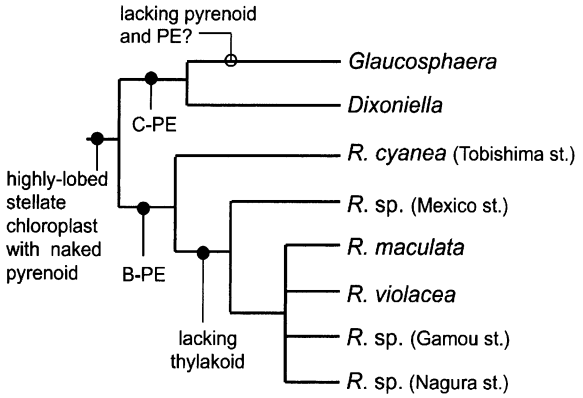


Figure 10. Structural and physiological characters are mapped onto the molecular phylogenetic tree inferred from 18SrDNA sequences. C-PE: Cyanophycean type of phycoerythrin; B-PE: Bangiophycean type of phycoerythrin; PE: phycoerythrin; lacking thylakoid: lacking thylakoid in the pyrenoid matrix.

thylakoids. No cytoplasmic space between the nucleus and the pyrenoid was found in *Rhodella* sp. (Mexico st.), while the nucleus of *R. cyanea* (Billard & Fresnel, 1986) and that of Tobishima st. (Fig. 6) were surrounded by cytoplasm and sometimes associated with dictyosomes.

The organellelar configuration and pyrenoid structure of Tobishima st. examined here are closely similar to those of *R. cyanea* (Billard & Fresnel, 1986). Initially we considered these to be conspecific. But a color difference exists between Tobishima st. (purple-red) and *R. cyanea* (blue-green). In our pigment analysis, Bangiophycean type of phycoerythrin was detected in the Tobishima st. *R. cyanea* was speculated to lack phycoerythrin from its blue-green cell color (Billard & Fresnel, 1986).

The cellular features of all species examined in this study and related species published so far are summarized in Table 2, for their taxonomic analysis. Species of *Rhodella* and *D. grisea* with a naked pyrenoid free from internal structures are primarily divided into two groups, depending mainly on the presence or absence of thylakoids in the pyrenoid matrix. If the presence of thylakoids in the pyrenoid matrix is adopted for the generic delimitation of *Rhodella*, *R. cyanea* has to be excluded from *Rhodella*. It is noteworthy that another structural characteristic of dictyosomes, which are located around the nucleus and associated with the nuclear envelope from their *cis* face, is common in *R. cyanea*, *D. grisea* and *Glaucosphaera vacuolata*.

The hypothetical scheme in Figure 8 based on Hara et al. (1989) and the results obtained here, including *Rhodella* species derived from *Porphyridium*, show two lineages: *Dixoniella*-lineage and *Rhodella*-lineage. As previously reported (Hara et al., 1989), the structural clines shown below are commonly recognized among Porphyridialean algal groups: (1) chloroplast; axile to peripheral, stellate to highly-lobed, presence or absence of pyrenoids, (2) pyrenoid; embedded to naked type, with or without thylakoids or tubular structures in the matrix, (3) nucleus; peripheral to central. Some of these are cited in Figure 8. However, lacking internal structures in the pyrenoid matrix (indicated by a horizontal arrow in Fig. 8) may give rise to a group intermediate between the two lineages.

Based on cellular features mentioned above, it is understandable that the genus *Rhodella* is delimited by possessing a pyrenoid without any internal structures in the matrix and dictyosomes directed and associated with ER by their *cis* faces (Table 2). In this sense, we have a problem because *R. cyanea* possesses convoluted thylakoids in the pyrenoid matrix which is common with *D. grisea*, and dictyosomes associated with nucleus which is common with *D. grisea* and *G. vacuolata* (Table 2).

For better understanding of the taxonomy and phylogeny of *Rhodella* and other related algae, the phylogenetic tree inferred from the 18SrDNA was constructed with *Porphyridium* and *Erythrotrichia* selected as the outgroups (Fig. 9). All species of *Rhodella* formed a clade whereas *D. grisea* and *G. vacuolata* made up another clade (Fig. 9). Consequently, members in each clade show monophyletic divergence. Our three undescribed species were phylogenetically supported to belong to the genus *Rhodella*. The generic autonomy of *Dixoniella* reported by Scott et al. (1992) was also supported. Based on the phylogenetic analysis, *R. cyanea* may remain in *Rhodella*, although it may also be excluded from *Rhodella*.

The results of cellular and pigment analyses obtained here and published elsewhere are mapped onto a simplified tree (Fig. 10) derived from Figure 9. All species examined in this study, except for *G. vacuolata*, commonly possess a highly-lobed stellate chloroplast with a naked pyrenoid, which is considered a symplesiomorphic character when compared with a stellate chloroplast having an embedded pyrenoid. The lack of a pyrenoid in *Glaucosphaera* is considered as autapomorphy. It is not so curious that the Cyanophycean type of phycoerythrin (C-PE) might occur in *D. grisea* but lacking in *G. vacuolata* (Table 2), while acquisition of Bangiophycean type of phycoerythrin (B-PE) might also occur at the branch indicated by a solid circle (marked with B-

PE, Fig. 10). Acquisition of B-PE can be understood as a synapomorphic character of *Rhodella* and lacking thylakoids from the pyrenoid matrix is also a synapomorphic character of *Rhodella*, except for *R. cyanea*.

The following opinion can be proposed based on these cellular and molecular phylogenetic analyses. There are two possibilities to delimit the genus *Rhodella*, *Rhodella sensu lato* and *Rhodella sensu stricto*. In the former case, the genus is defined by possessing B-PE and includes all species of *Rhodella* published so far, except *R. grisea*/*R. reticulata* which was transferred to *Dixoniella* as *D. grisea*. In the latter case, *Rhodella* is defined by possessing a pyrenoid free from any internal structures in the matrix and dictyosomes associated with ER. It includes all species of *Rhodella* except *R. cyanea*. These two possibilities are both supported by the molecular phylogenetic analysis (Fig. 9).

Although it is necessary to discuss the taxonomic position of *R. cyanea* and the question of whether Tobishima strain is conspecific with *R. cyanea* or not, unfortunately, we had no chance to examine the type strain used by Billard & Fresnel (1986). It is only after investigations of the pigment and molecular phylogenetic analyses, using the type strain of *R. cyanea*, could the generic delimitation of *Rhodella* and the taxonomic position of *R. cyanea* be finally confirmed.

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References

Billard, C. & J. Fresnel, 1986. Plant systematics. *Rhodella cyanea* nov. sp., a new unicellular Rhodophytan alga. C. R. Acad. Sc. Paris, t. 302, Série III: 271–276.

Broadwater, S. T. & J. L. Scott, 1994. Ultrastructure of unicellular red algae. In Seckbach, J. (ed.), Evolutionary Pathways and Enigmatic Algae: *Cyanidium caldarium* (Rhodophyta) and Related Cells. Kluwer Academic Publishers, Dordrecht: 215–230.

Broadwater, S. T., J. L. Scott, S. P. A. Gross & B. D. Saunders, 1995. Ultrastructure of vegetative organization and cell division in *Glaucosphaera vacuolata* Korshikov (Porphyridiales, Rhodophyta). Phycologia 34: 351–361.

Deason, T. D., G. L. Bulter & C. Rhyne, 1983. *Rhodella reticulata* sp. nov., a new coccoid rhodophytan alga (Porphyridiales). J. Phycol. 19: 104–111.

Evans, L. V., 1970. Electron microscopical observations on a new red algal unicell, *Rhodella maculata* gen. nov., sp. nov. Br. phycol. J. 5: 1–13.

Fresnel, J., C. Billard, F. Hindák & B. Pekárková. 1989. New observations on *Porphyridium griseum* Geitler = *Rhodella grisea* (Geitler) comb. nova (Porphyridiales, Rhodophyta). Pl. Syst. Evol. 164: 253–262.

Hara, Y. & M. Chihara, 1985. Ultrastructure and taxonomy of *Fibrocapsa japonica* (Class Raphidophyceae). Arch. Protistenk. 130: 133–141.

Hara, Y., T. Nagumo & S. Kato, 1989. Microalgal flora of mangrove forests in Japan. Proceedings of the 5th International Symposium on Microbial Ecology (ISME5): 292–296.

Hasebe, M. & K. Iwatsuki, 1990. *Adiantum capillus-veneris* Chloroplast DNA clone bank as useful heterologous probes in the systematics of the leptosporangiate ferns. Am. Fern J. 80: 20–25.

Hasebe, M., T. Omori, M. Nakazawa, T. Sano, M. Kato & K. Iwatsuki, 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc. Natl. Acad. Sci. U.S.A. 91: 5730–5734.

Koller, K. P. & W. Wehrmeyer, 1975. B-phycoerythrin from *Rhodella violacea;* characterization of two isoproteins. Arch. Microbiol. 104: 255–261.

Nakayama, T., S. Watanabe, K. Mitsui, H. Uchida & I. Inouye, 1996. The phylogenetic relationship between the Chlamydomonadales and Chlorococcales inferred from 18SrDNA sequence data. Phycol. Res. 44: 47–55.

Okaichi, T., S. Nishino & Y. Imatomi, 1982. Collection and mass culture. In Bull. Jap. Soc. Sci. Fish. (ed.), Toxic Phytoplankton Occurrence, Mode of Action and Toxins. Kôseisha-Kôseikaku, Tokyo: 23–34 [in Japanese].

Olsen, G. J., H. Matsuda, R. Hagstrom & R. Overbeek, 1994. fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. CABIOS 10: 41–48.

Patrone, L. M., S. T. Broadwater & J. L. Scott, 1991. Ultrastructure of vegetative and dividing cells of the unicellular red algae *Rhodella violacea* and *Rhodella maculata*. J. Phycol. 27: 742–753.

Ragan, M. A., C. J. Bird, E. L. Rice, R. R. Gutell, C. A. Murphy & R. K. Singth, 1994. A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small-subunit rRNA gene. Proc. Natl. Acad. Sci. U.S.A. 91: 7276–7280.

Schornstein, K. L. & J. Scott, 1982. Ultrastructure of cell division in the unicellular red alga *Porphyridium purpureum*. Can. J. Bot. 60: 85–97.

Scott, J. L., S. T. Broadwater, B. D. Saunders, J. P. Thomas & P. W. Gabrielson, 1992. Ultrastructure of vegetative organization and cell division in the unicellular red alga *Dixoniella grisea* gen. nov. (Rhodophyta) and a consideration of the genus *Rhodella*. J. Phycol. 28: 649–660.

Swofford, D. L., 1993. PAUP; Phylogenetic Analysis Using Parsimony, version 3.1.1. Computer program and manual. Illinois Natural History Survey, Champaign, Illinois.

Wehrmeyer, W., 1971. Elektronmikroskopische Untersuchung zur Feinstructur von *Porphyridium violaceum* Kornmann mit Bemerkungen über seine taxonomische Stellung. Arch. Mikrobiol. 75: 121–139.