J. Plant Biol. 37(4) : 403~410 (1994)

Taxonomic Investigation of *Erythroglossum minimum* Okamura and *Sorella repens* (Okamura) Hollenberg (Delesseriaceae, Rhodophyta)

Kim, Gwang Hoon*, Jung Hyun Oak[1] and In Kyu Lee[1]

Department of Biology, Kongju National University, Kongju 314-701, Korea; and

[1]Department of Biology, Seoul National University, Seoul 151-742, Korea

The taxonomic criterion between *Erythroglossum* and *Sorella* was reappraised based on Korean plants of *E. minimum* Okamura and *S. repens* (Okamura) Hollenberg. Development of the reproductive organs was observed from field collected plants as well as cultured plants. The *Polyneura*-type procarp which comprised one group of sterile cells and two carpogonial branches arising from a supporting cell was observed in both species. Distribution of tetrasporangial sori was irregular in both species, so it is not relevant to distinguish *Sorella* from *Erythroglossum* by the characteristic of tetrasporangial distribution. *E. minimum* and *S. repens* were very similar in vegetative form, but were reproductively isolated. Chromosome numbers were given as n=ca. 30 in *S. repens* and n=ca. 41 in *E. minimum*, respectively.

Keywords : *Sorella, Erythroglossum,* taxonomy, chromosome, *Polyneura*-type procarp

The genus *Erythroglossum*, Nitophylloideae, Delesseriaceae was established by J. Agardh (1898), and about ten species are enumerated in it. Hollenberg (1943) established the genus *Sorella* from *Erythroglossum*, distinguishing it by the characters of relatively narrow thallus and tetrasporangial sori occurring at the median portion of branches and branchlets, in contrast to marginal tetrasporangial sori in *Erythroglossum*. The distribution of tetrasporangial sori, however, was regarded as an unstable character in Delesseriaceae (Yamada, 1935; Mikami, 1970, 1976). Yamada (1971) studied a detailed development of reproductive structures of *S. repens* (Okamura) Hollenberg and reported that the procarp comprised one group of sterile cells and two carpogonial branches arising from a supporting cell. Such a procarp, called *Polyneura*-type, was known in *Polyneura* (Kylin, 1956). He suggested that a *Polyneura*-type procarp would be adopted as a key character to separate *Sorella* from *Erythroglossum*, if the latter exhibit *Myriogramme*-type as mentioned by Kylin (1933) in *E. undulatissimum*. Supporting Yamada's suggestion (1971), Stewart (1977) reported that the other species of *Sorella, S. delicatula* var. *californica* Hollenberg and *S. pinnata* Hollenberg also had a *Polyneura*-type procarp.

Mikami (1976, 1977) reported that *Erythroglossum minimum* Okamura and *E. pinnatum* Okamura also had a *Polyneura*-type procarp, and confirmed that the tetrasporangial sori occurred at the marginal portion of branches and branchlets, as known by a diagnostic character of the *Erythroglossum*. Recently, Yoshida and Mikami (1991) observed a *Polyneura*-type procarp in *E. pulchrum* Yamada, and combined it as *S. pulchra* (Yamada) Yoshida et Mikami. Therefore, the taxonomic criteria of both genera are still in debate.

In this study, we re-evaluated taxonomic characters which distinguish *Sorella* from *Erythroglossum* by investigating the development of reproductive and vegetative structures of Korean *S. repens* and *E. minimum* plants in the field and in culture.

*Corresponding author: Fax +82-416-50-8504

Table 1. Relative abundance of female, male and tetrasporic plants of *E. minimum* in Korea

Month	No. examined	Female	Male	Tetrasporic	Sterile
Mar.-Apr.	91	0%	0%	69%	31%
Jul.-Aug.	137	9	1	65	25
Nov.-Dec.	209	25	5	59	11

MATERIALS AND METHODS

The plants of *S. repens* and *E. minimum* were collected from several sites of southern coasts of Korea during 1985 and 1994. The materials were preserved in 5% formalin-seawater and stained with 1% aniline blue for microscopic observation of reproductive structure. Unialgal culture was carried out with spores and excised apices of thallus. They were maintained in PES medium (Provasoli, 1968) at 10, 15, 20℃, under 10-20 $\mu E \cdot m^{-2} \cdot s^{-1}$ with 16 : 8, 12 : 12, and 8 : 16 LD. The medium was changed in every two weeks. For chromosome study the plants were kept at 15℃, 16 : 8 LD and 10 $\mu E \cdot m^{-2} \cdot s^{-1}$ for a while and fixed for 12-24 h in ethanol-acetic acid solution, mordanted for 15 min in iron-alum-haematoxilin solution, stained with 2% acetocarmine or 1% acetic orcein, then squashed and sealed with aceto-gelatin.

RESULTS

Erythroglossum minimum Okamura

Field observations: Plants of *E. minimum* were found at shaded places in the intertidal to the subtidal zones along the coasts of Korea, associated with several benthic organisms rather than being restricted to a particular substrate, and often collected with *S. repens*. The warmer season in Korea, August-October, seemed to be most favorable for growth. The relative abundances of female, male and tetrasporic plants are shown in Table 1. The tetrasporic plants were found through all the months, while female and male plants were absent in March-April, rare in July-August, and numerous in November-December.

The plants were small, erect, 1-3 cm high and 0.4-1.5 mm broad at median portion of main branches. The branches developed once or 2-4 times pinnately, partly alternately to pseudodichotomously, and narrow at base (Fig. 1). The thallus was monostromatic near the margin and younger portion, but became 5-8 layers in the lower portion. A midrib was present, but lateral veins were lacking. Large apical cell was observed in acuminate apices, and the intercalary cell divisions occurred in the primary cell row (Fig. 2A-D).

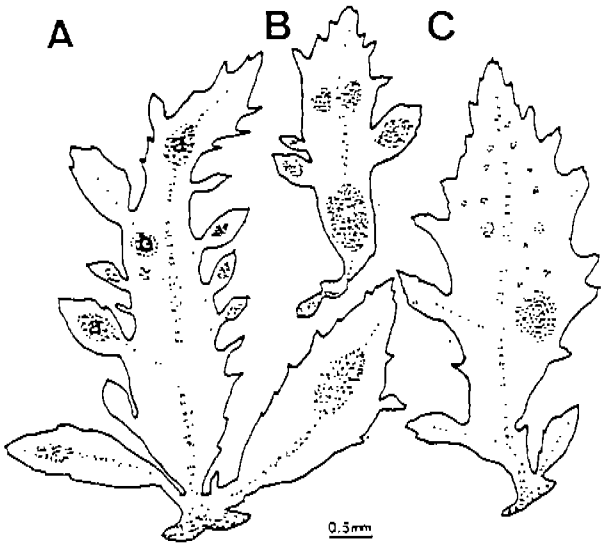

Fig. 1. Development of reproductive structures in *E. minimum* Okamura. A, tetrasporic plant with *Sorella*-type tetrasporangial sori and *Erythroglossum*-type tetrasporangial sori; B, male plant with spermatangial sori; C, Female plant with procarps and cystocarp.

Development of tetrasporangia: Tetrasporangial sori occurred in both the median and marginal portions of the thallus, forming round to elliptical patches (Fig. 1A). According to Hollenberg (1943), the occurrence of tetrasporangial sori in median portion was the most important diagnostic character of the genus *Sorella*. However, most of the tetrasporic plants investigated formed the sori in median portion of the branches, and only 21 plants (7%) among 298 mature tetrasporic plants developed them in the marginal portion and the rest in the median portion or the both portions together.

In development of tetrasporangia a vegetative cell divided periclinally into three, one central and two pericentral cells. Both pericentral cells cut off small surface cells and remaining with a cortical cell, respectively. These central and cortical cells produced

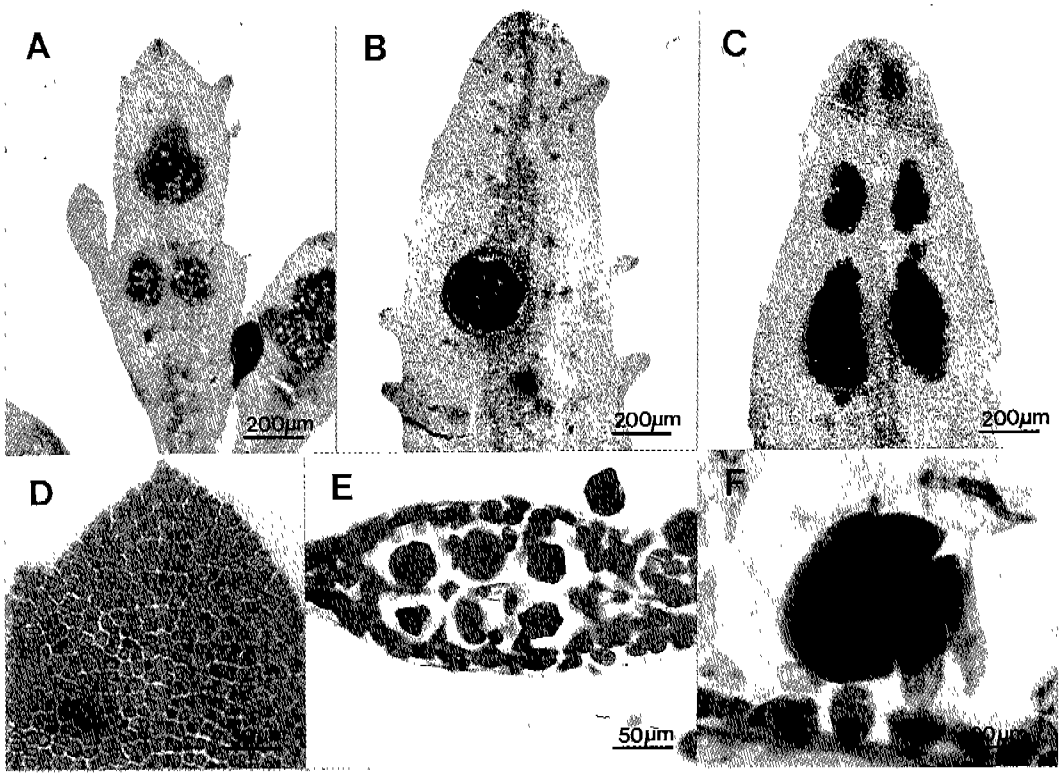

Fig. 2. Development of vegetative and reproductive structures in *E. minimum*. A, a tetrasporic plants with tetrasporangial sori in the median and the lateral portion of thallus; B, female gametophyte with cystocarp and procarps; C, male gametophyte with spermatangial sori; D, enlarged view of apical portion of thallus; E, transverse sectional view of tetrasporangial sori; F, tetrasporangium.

tetrasporangial initials together, which grow and divide tetrahedrally (Fig. 2E). A mature tetrasporangium was spherical and about 50 μm in diameter (Fig. 2F).

Development of spermatangia: The spermatangial sori developed from marginal and median portions on both surfaces of the branches, and extended to irregular patches (Fig. 1B, 2C). In development of spermatangia, each vegetative cell divided periclinally into three, like as in tetrasporangium formation. The pericentral cells thus derived divided repeatedly into many small cells anticlinally, which became the spermatangial mother cells, respectively (Fig. 3A-C). Two to three small spermatangia were protruded subterminally from the outer margin of the mother cell successively, and cut off obliquely to form young spermatangia (Fig. 3D). The spermatangium first appeared as a narrow beak-like protrusion from the mother cell, increased in size, and was divided later by an oblique annular ingrowth of the wall. The young spermatangia were embedded by the gelatinous material which was easily observed by staining with 2% aniline blue solution. A mature spermatangium was 3 μm in diameter.

Development of procarp and cystocarp: The procarps were scattered over upper portion of the branches (Fig. 1C). An initial cell of the procarp was distinguished easily from neighboring vegetative cells by its large size and deep staining. When the cell grew to about two times, it divided periclinally into three, one central and two pericentral cells. Each pericentral cell could develop the procarp by successive divisions, as follows: At first, one of the pericentral cells cut off a sterile cell toward the surface, then divided anticlinally forming a mother cell of the first carpogonial branch (Fig. 3E). In addition, it cuts off again anticlinally another mother cell of the second carpogonial branch (Fig. 3F). The rest pericentral cell became the supporting cell (Fig. 3G). Each of the mother cells developed into a four-cell-

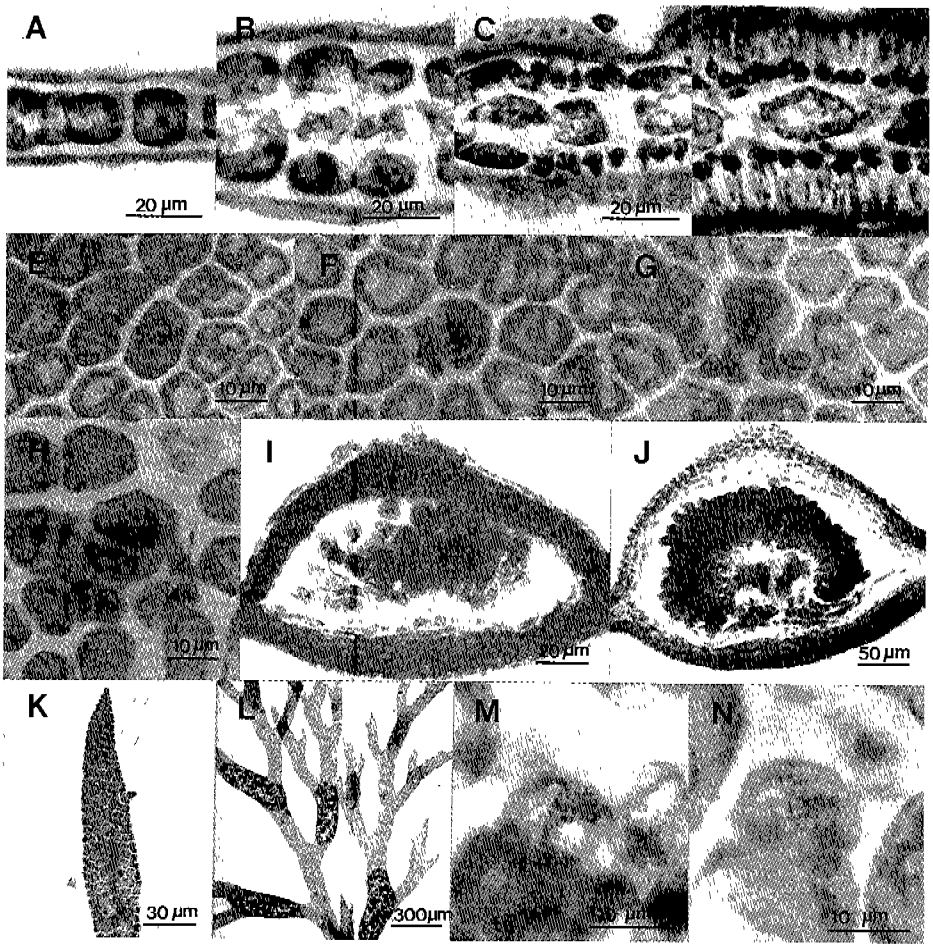

Fig. 3. Development of vegetative and reproductive structures in *E. minimum* and *S. repens*. A-D, transverse sectional view of spermatangial development in *E. minimum*; E-H, development of procarp in surface view; I, young cystocarp showing gonimoblast; J, mature cystocarp; K, tetraspore germling of *E. minimum* three weeks after germination; L, tetrasporophyte of *S. repens* with developed tetrasporangial sori; M, chromosomes of *S. repens*; N, chromosomes of *E. minimum*.

ed carpogonial branch with a trichogyne (Fig. 3H). Sometimes, the second carpogonial branch was not fully developed even after the first one was completed. During development of the procarp, the sterile cell already cut off from the pericentral cell divided into two to three cells, which were stained less deeply with aniline blue solution than the supporting cell. When the carpogonial branches were completed, the sterile cells, four to six in number, covered the carpogonial branches and supporting cell. A mature procarp, in surface view, usually had a characteristic shape. The supporting cell was located between two carpogonial branches, which were bent toward the apex of thallus.

After fertilization, both the trichogynes disappeared, and the supporting cell enlarged in size and cut off an auxiliary cell from one side. After this stage, the development of cystocarp could not be discerned from surface of the thallus. In transverse section of the young cystocarp, an irregular shaped large fusion cell, formed by procarpic cells, gave rise to many early cells of gonimoblast covered with gelatinous material, except for one or two terminal cells (Fig. 3I). These naked terminal cells produced carposporangia in chain. Meanwhile, the sterile cells enlarged and the surrounding vegetative cells around gonimoblast divided into small cells to form a pericarp. A mature cystocarp became hemispherical with an ostiole at the apex. It was about 400-480 μm broad and 150-200 μm high (Fig. 3J).

Laboratory culture: The laboratory culture of *E. minimum* started with the tetrasporic plants collected at Wando in November, 1985 and the carposporic plants collected at Sungsan, Cheju Island in February, 1986. The spores obtained by unialgal culture hardly attached to the cover glass, and only a few of them attached to grow into four to six celled sporelings within two days. After ten days, the lateral pericentral cells were formed, and two weeks later the median pericentral cells were formed (Fig. 3K). Male and female reproductive structures appeared after six weeks. They were crossed successfully to form a cystocarp. The development of reproductive structures and post fertilization process was identical with field materials. Cystocarps matured in four weeks after fertilization. Released carpospores developed into mature tetrasporophytes in eight weeks after germination. Therefore, it required about six months to complete a typical *Polysiphonia*-type life history in laboratory culture. When tetrasporic plants, developing the sori typically in the marginal portions of thallus, were grown under various culture conditions, most of the plants developed tetrasporangial sori in the median portion of branch, and only a few of them (<5%) developed in the marginal portion.

Sorella repens (Okamura) Hollenberg

Basionym: *Erythroglossum repens* Okamura (1929)

Field observations: The plants of *S. repens* grew mainly at shady places in intertidal zone, associated with several benthic organisms, and sometimes were collected together with *E. minimum* or *Acrosorium yendoi* Yamada. They also grew most luxuriantly during the warmer season, August-October. The tetrasporic plants were found through the year, while gametophytic plants were rare in July-August, and numerous in November-December in Korea.

The thallus was small, erect but sometimes prostrate by emitting disc-like haptera from both marginal surfaces in lower portion. Main branches were broadly expanding in a dichotomo-alternate manner with pinnate branchlets. The tetrasporic plants were 1.0-3.0 cm high and 0.2-1.2 mm broad at lower portion of a thallus, while the gametophytic plants are 0.5-1.0 cm high and 0.5-0.6 mm broad in maximum.

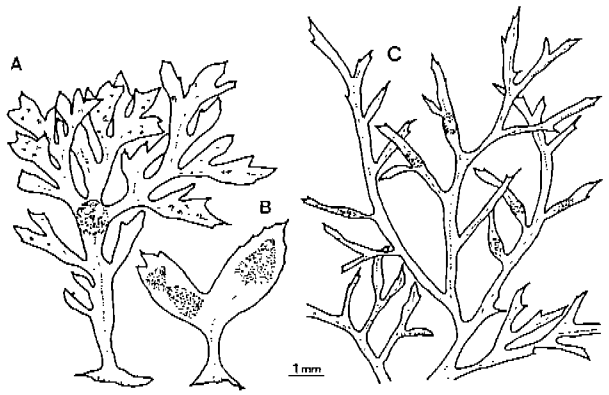

Fig. 4. Development of reproductive structures in *S. repens* (Okam.) Hollenberg. A, female plant with cystocarp; B, male plant with spermatangial sori; C, tetrasporic plant with tetrasporangial sori.

Sometimes, in gametophytes, the thallus became gradually narrower toward the base to form a stipe. A midrib was present but lateral veins were lacking. An apical cell was present in the acuminate apices. Intercalary cell divisions occurred in the primary cell row, as seen in *E. minimum*.

Development of reproductive structures: Tetrasporangial sori occurred mainly in median portions of the branches (Fig. 3L, 4C). However, the sori developed in marginal portion were not rare. Most of the tetrasporic plants investigated formed the sori in median portions of the branch, but 32 plants (9%) among 342 mature tetrasporic plants developed the sori in the marginal portions. The procarps scattered over the upper portion of the thallus (Fig. 4A).

Laboratory culture: Unialgal culture of *S. repens* started with the tetrasporic plants collected at Kangneung in February, 1985. The development of reproductive structure and morphogenesis were basically same as *E. minimum*. A *Polysiphonia*-type life history was shown in laboratory culture, which required eight month to complete. The development of reproductive structures was identical with field materials. The reciprocal crosses between *S. repens* and *E. minimum* were not successful.

Chromosome investigation: Gametophytic plants, showing typical forms of both species grown in the laboratory, were used for chromosome investigation.

To secure the exact chromosome number and the absence or presence of supernumerary chromosomes or fragments, three plants were counted from each of six different thalli. The shape of chromosomes was dot-like in both species, and the numbers were n=ca. 30 for *S. repens* (Fig. 3M), and n=ca. 41 for *E. minimum* (Fig. 3N).

DISCUSSION

The Korean plants of *E. minimum* and *S. repens* agreed well with the species originally described by Okamura (1929), in height, breadth and branching pattern of thalli. The developments of reproductive structures, tetrasporangia, spermatangia, procarps and cystocarps, were basically similar in both species. Each of the procarps comprised one group of sterile cells and two carpogonial branches arising from a supporting cell. Such a procarp, called *Polyneura*-type, was known only in *Polyneura* (Kylin, 1956) and *Sorella* (Yamada, 1971; Stewart, 1977). The procarpic structures of *Erythroglossum* was reported as *Myriogramme*-type for *E. undulatissimum* (Kylin, 1933). Yamada (1971) suggested that the structure of procarps would be adopted as one of the diagnostic character to separate the genus *Sorella* from *Erythroglossum*. Mikami (1976, 1977), however, observed the same *Polyneura*-type procarp in *E. minimum* and *E. pinnatum* Okamura. Mikami (1987) also observed *Sorella*-type tetrasporangial sori in the type specimen of *E. pulchrum* Yamada. Based on the results, Yoshida and Mikami (1991) transferred the species to the genus *Sorella*.

In this study, we tried to elucidate the taxonomic circumscription between *Sorella* and *Erythroglossum* by analysing validity and stability of characters which distinguished the both. As a result, there was no significant difference in gross morphology and reproductive structures. The distribution of tetrasporangial sori, one of the most significant characters adopted by Hollenberg (1943) to distinguish *Sorella* from *Erythroglossum*, was found to be very unstable character in both species, as previously reported in other genus, *Acrosorium*, of the Delesseriaceae (Yamada, 1935; Mikami, 1970).

Thus, the simplest way to solve this problem will be clarifying the nature of procarps in type species of both the genera. As to the type species, however, it is far more delicate in situation. The type species of the genus *Sorella*, *S. delicatula* (Gardner) Hollenberg, was first described by Gardner (1926) as *E. delicatula* based on a drift specimen of 9 cm height from southern California. With the same specimen, Hollenberg (1943) established the genus *Sorella*, and he described much smaller but otherwise similar plants collected from low intertidal habitats in Orange County, California, as a variety of the species. Dawson (1962) placed this variety in synonymy of the species, on the stated assumption that the size of the large type specimen described originally by Gardner (1926) as *E. delicatula* was related to an environmental effect, and that similarly large plants would be found in deep water as Hollenberg (1943) had suggested. Later, Abbott and Hollenberg (1976) agreed to it. As it was, Stewart (1977) reported that the data, based on the careful subtidal collecting by SCUBA diving for two years in type locality and various sites, offered no support for Dawson's suggestion that the large specimen would be a deep water form of the species. We also could not find any significant differences between the intertidal and subtidal plants in morphology and dimension of thallus. However, when fully grown, the culture plants of *S. repens* were longer (upto 5 cm) than field collected plants (1-3 cm). Therefore, the large type specimen of *S. delicatula* would not be a deep water form, or it could be resulted from some favourable condition for growth of that species. From this point of view, we agree with Dawson's suggestion. In addition, Hollenberg (1943) and Yamada (1971) suggested that *S. repens* was similar to *S. delicatula* or *S. delicatula* var. *californica*. We could not find any significant differences between the two species, so that re-examination of the relationship between them by crossing test or chromosome analysis is necessary.

As to the type of the genus *Erythroglossum*, Kylin (1924) first designated *E. schousboei* as the lectotype species but later (1956) selected *E. bipinnatifidum*. Examining several dried specimens of *E. bipinnatifidum* from Chile, identified by Etcheverry, Mikami (1979) suggested that *E. bipinnatifidum* belonged to *Branchioglossum* in apical organization and reproductive characteristics. Wynne (1983) transferred *E. bipinnatifidum* to *Branchioglossum* based on the result of Mikami (1979). However, as Mikami (1979) himself mentioned, his study was not based on the type

specimen and, moreover, his observation was not coincident with original description of that species (Kylin, 1924). Thus, the procarpic character as well as the type species of the genus *Erythroglossum* is still under discussion.

As a result, in order to clarify the relationship between *Sorella* and *Erythroglossum,* the main character of the former, the distribution of tetrasporangial sori, should be re-evaluated. According to Yamada (1971) and Mikami (1979, 1987) as well as the present investigation, such a character is rather variable and is overlapped among species, and is not relevant to distinguish *Sorella* from *Erythroglossum*. So far, any relevant taxonomic characters are not found, the genus *Sorella* Hollenberg (1943) should be reduced as a synonymy of *Erythroglossum.* Otherwise, the concept of genus *Sorella* should be emended based on the character of procarp to include *E. minimum* and *E. pinnatum,* bearing a *Polyneura*-type procarp, by confirming that the type species of *Erythroglossum* bears a *Myriogramme*-type procarp.

ACKNOWLEDGMENTS

This work was supported by a grant from Korean Ministry of Education, BSRI 93-410 to I.K. LEE.

LITERATURE CITED

Abbott, I.A. and G.J. Hollenberg. 1976. Marine Algae of California. Stanford University Press, Palo Alto, pp. 647-650.

Agardh, J.G. 1898. Species genera et ordines algarum. Vol. III. Parts 3, De dispositione Delesseriearum mantissa algologica, Lund. pp. 1-239.

Dawson, E.Y. 1962. Marine red algae of Pacific Mexico. VII. Ceramiales: Ceramiaceae, Delesseriaceae. *Allan Hancock Pacific Exped.* **26**: 1-207.

Dixon, P.S. 1973. Biology of the Rhodophyta. Oliver and Boyd, Edinburgh, pp. 89-93.

Gardner, N.L. 1926. New Rhodophyccae from the Pacific Coast of North America. I. *Univ. Calif. Publ. Bot.* **13**: 205-226.

Hollenberg, G.J. 1943. New marine algae from Southern California. II. *Am. J. Bot.* **30**: 571-579.

Kylin, H. 1924. Studien uber die Delesseriaceen. *Lunds Univ. Arsskr. N.F.* **2**: 1-111.

Kylin, H. 1933. On three species of Delesseriaceae from New Zealand. *Trans. New Zeal. Inst.* **63**: 109-111.

Kylin, H. 1956. Dic Gattungen dcr Rhodophyceen. CWK Gleerups Forlag, Lund. pp. 1-673.

Mikami, H. 1970. On the reproductive organs in *Acrosorium yendoi* Yamada. *Bull. Jpn. Soc. Phycol.* **18**: 60-66.

Mikami, H. 1976. New knowledge on *Erythroglossum minimum* Okamura (Rhodophyceae, Delesseriaceae) from Japan. *Bull. Jpn. Soc. Phycol.* **24**: 81-86.

Mikami, H. 1977. On *Erythroglossum pinnatum* Okamura (Rhodophyceae, Delesseriaceae) from Japan. *Bull. Jpn. Soc. Phycol.* **25**, Suppl.: 143-147.

Mikami, H. 1979. On *Erythroglossum bipinnatifidum* (Rhodophyceae, Delesseriaceae) from Chile. *Jpn. J. Phycol.* **27**: 35-38.

Mikami, H. 1987. On *Erythroglossum pulchrum* Yamada and *Hypoglossum sagamianum* Yamada (Rhodophyta, Delesseriaceae). *Jpn. J. Phycol.* **35**: 124-129.

Okamura, K. 1929. Icons of Japanese Algae. VI. Uchida Rokakuho Publishing House, Tokyo, pp. 9-10, 88-93.

Papenfuss, G.F. 1937. The structure and reproduction of *Claudea multifida, Vanvoorstia spectabilis* and *V. coccinea. Symb. Bot. Upsal.* **2**: 1-66.

Provasoli, L. 1968. Media and prospects for the cultivation of marine algae. *In* Culture and Collection of Algae, A. Wantabe and A. Hattori (eds.). Proc. US-Japan Conf. Hakone Sep. 1966. Jpn. Soc. Plant Physiol., pp. 63-75.

Stewart, J.G. 1977. Morphology of *Sorella* spp. in natural habitats and under labortory conditions. *Bull. Soc. Calif. Acad. Sci.* **76**: 5-15.

Wagner, F.S. 1954. Contribution to morphology of the Delesseriaceae. *Univ. Calif. Publ. Bot.* **27**: 279-346.

Wynne, M.J. 1983. The current status of genera in the Delesseriaceae (Rhodophyta). *Bot. Mar.* **26**: 437-450.

Wynne, M.J. and K. Daniels. 1966. *Cumathamnion*, a new genus of the Delesseriaceae. *Phycologia* **6**: 13-28.

Yamada, I. 1971. Observations on *Sorella repens* (Okamura) Hollenberg (Rhodophyta) in Japan, especially on the development of the reproductive organs. *Phycologia* **10**: 189-198.

Yamada, Y. 1935. Notes on some Japanese algae. IV. *Sci. Pap. Inst. Alg. Res., Hokaido Imp. Univ.* **1**: 124-125.

Yoshida, T. and H. Mikami. 1991. *Sorella pulchra* (Yamada) comb. nov., based on *Erythroglossum pulchrum* Yamada (Delesseriaceae, Rhodophyta). *Jpn. J. Phycol. (Sorui)* **39**: 123-129.

(Received October 17, 1994)

韓國產 紅藻 *Erythroglossum minimum* Okamura와 *Sorella repens* (Okamura) Hollenberg의 分類

金 光 勳* · 玉 政 玹[1] · 李 仁 圭[1]
公州大學校 自然科學大學 生物學科, [1]서울大學校 自然科學大學 生物學科

적 요

한국 연안에 생육하는 *Erythroglossum minimum*과 *Sorella repens*를 대상으로 실내배양 및 염색체 관찰과 형태적 연구를 통해 식별형질들을 검토하고 이들의 분류학적 타당성을 고찰하였다. 한국산 *E. minimum*과 *S. repens*는 모두 특이한 *Polyneura*형 전과체 구조를 가지며 속의 식별형질인 사분포자낭반의 위치에 변이가 심할 뿐 아니라 종간의 중간 형태가 관찰되는 등 종의 한계를 규정하기 어려운 문제점을 갖고 있다. 본 연구에서는 다음과 같은 결론에 도달하였다. (1) 한국산 *E. minimum*은 여러 가지 식별형질에서 *Sorella*속의 특성과 더 잘 부합되며, (2) 사분포자낭반의 위치는 두 종에서 모두 상당한 변이가 관찰되어서, 식별형질로서의 채용이 재고되어야 한다. (3) 교배실험의 결과 이들은 생식적으로 격리되어 있으며, 염색체의 수가 *E. minimum*은 n=ca. 41, *S. repens*는 n=ca. 30인 것으로 볼 때 두 종은 뚜렷이 독립된 분류군임을 확인할 수 있었다.

주요어 : *Sorella*, *Erythroglossum*, 분류, 염색체, *Polyneura*형 전과체

*교신저자 : Fax (0416) 50-8504