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Kazuyuki Miyaji: The occurrence of *Rhizoclonium riparium* and *R. tortuosum* (Chlorophyceae) on the coast of Hokkaido, Japan.

Key Index Words: Chaetomorpha—*Cladophoraceae*—Lola—Rhizoclonium riparium—Rhizoclonium tortuosum—*taxonomy*
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The genus *Rhizoclonium* is one of the simplest of the Cladophoraceae, but this simple structure has led to taxonomic confusion. The relationship between *Rhizoclonium implexum* (Dillwyn) Kützing, *R. riparium* (Roth) Kützing ex Harvey and *R. tortuosum* (Dillwyn) Kützing is particularly unclear. To resolve this confusion, Koster (1955) revised the taxonomy of the genus by comparing specimens of various species. She concluded that *R. implexum, R. kochianum* Kützing and *R. kerneri* Stockmayer, and also *R. riparium* and *R. tortuosum* are the same species. She placed *R. tortuosum* in synonymy with *R. riparium* f. *validum* Foslie, since the only recognizable difference between the two entities was cell width. Also, Scagel (1966) speculated that the differences in cell width between the two taxa may be a result of environmental factors, and suggested that the two species were synonymous; he also did not recognize the forma *validum*. By contrast, *R. tortuosum* is sometimes treated as a species of the genus *Lola* (Hamel 1930; Chapman 1952, 1956) or the genus *Chaetomorpha* (Kützing 1845, De-Toni 1889, Børgensen 1902, Jónsson 1903, Kornmann 1972, Kornmann and Sahling 1977).

In Hokkaido and adjacent waters, only *R. tortuosum* (Japanese name "Naga-motsure") has been reported (Kawabata 1936, Nagai 1940, Yamada and Tanaka 1944, Tokida 1954, Chihara 1972). However, I have found that *R. riparium* (Japanese name "Hoso-nedashigusa") also occurs in Hokkaido. The present study attempts to elucidate whether the two entities distributed in Japan are distinct species or the result of differing environmental conditions.

The specimens used in this study were collected at the following localities: *Rhizoclonium riparium*; Tokkarisho, Muroran (March 10, 1974), Daikoku Islet, Akkeshi (August 17, 1974), Kiritappu, Nemuro (July 25, 1972); *R. tortuosum*; Muroran (August 17, 1974), Harutachi, Hidaka (July 21, 1974), Kiritappu, Nemuro (July 25, 1972). They were preserved in 10% Formalin in sea water. Part of the liquid-preserved material was dried on herbarium sheets. The specimens examined in the present work are deposited in the Herbarium of Faculty of Science, Toho University. *Rhizoclonium riparium* occurs on rock from the upper littoral zone to the supralittoral zone where there is freshwater run-off due to rain or melting snow. This species is sometimes associated with *Blidingia minima* (Nägeli ex Kützing) Kylin. *Rhizoclonium tortuosum* is usually entangled with other algae such as *Sargassum* spp., *Cystoseira* spp., *Neorhodomela aculeata* (Perestenko) Masuda, *Tichocarpus crinitus* (S.G., Gmelin) Ruprecht ex Middendorff, which occur in the middle and lower littoral zone. Plants sometimes grow on *Corallina pilulifera* Postels et Ruprecht. However, *R. riparium* is never entwined with other algae. *Rhizoclonium riparium* grows in entangled masses of uniseriate filaments with numerous short, tapering rhizoidal branches. *Rhizoclonium tortuosum* may also be found as entangled masses of uniseriate filaments, which, however, have no intercalary rhizoidal branches, and are sometimes twisted and contorted. Filaments of *R. riparium* are (17.5–)20–25(–35) μm broad and (0.7–)1–1.5(–4.5) times as long as broad. Filaments of *R. tortuosum* are (30–)35–40(–60) μm broad with cells (1.5–)3–4(–8.0) times as long as broad. Frequency curves of

occurrence of various cell widths of the two entities from the six collection sites show that the cell width of each entity is roughly the same (Fig. 1A). However, the range of variation and the peak of the frequency curve differ slightly with each collecting locality, and the frequency curves of the two entities do not overlap (Fig. 1A). Frequency curves of the occurrence of various cell width to length ratios in each entity are shown in Fig. 2. These figures show that cell ratio is also roughly similar in the same entity except for plants of *R. riparium* from Kiritappu, and that the frequency curves of the occurrence of various cell ratios between the two entities overlap somewhat (Fig. 2). These data on cell width and the cell ratio show also that variation in the above features is usually slight in *R. riparium*, but more pronounced in *R. tortuosum*.

Aceto-carmine was used for counting numbers of the nuclei in a cell. The materials used for counting nuclear numbers were the same as liquid-preserved materials for morphological observation. Nuclei of *R. riparium* are located in the center of a cell (Fig. 3A). A histogram of the occurrence of nuclear number in a cell shows that the number is generally one or two in all collection sites; numbers greater than two were observed only infrequently, and the largest number seen being eight (Fig. 4A). The number most frequently occurring at every collection site is two, except for plants from Kiritappu, which had only one nucleus (Fig. 4A). Nuclei of *R. tortuosum* are scattered evenly near the periphery of the cell (Fig. 3B), and the number in each cell varies from eight to eighty. A histogram of occurrence of nuclear number in a cell of *R. tortuosum* shows that nuclear number is generally between 20–40 at every collection site (Fig. 4B). *Rhizoclonium tortuosum* samples show some tendency toward increased nuclear number in the cells as cell width increases (Fig. 1A, 4B).

For culture studies, plants of *Rhizoclonium riparium* were collected at Tokkarisho, Muroran on March 10, 1974 and Daikoku Islet, Akkeshi on August 17, 1974. In addition, plants of *R. tortuosum* were collected at Harutachi, Hidaka on July 17, 1974. These plants were rinsed with filtered seawater, and separated with a micropipette into small pieces, each containing three to five cells. Each piece was washed three times in autoclaved seawater. After washing, they were placed in test tubes with screw caps (2 cm × 18 cm) containing 10 ml of ESP medium (Provasoli 1966). After about a month, the plants that were not contaminated with

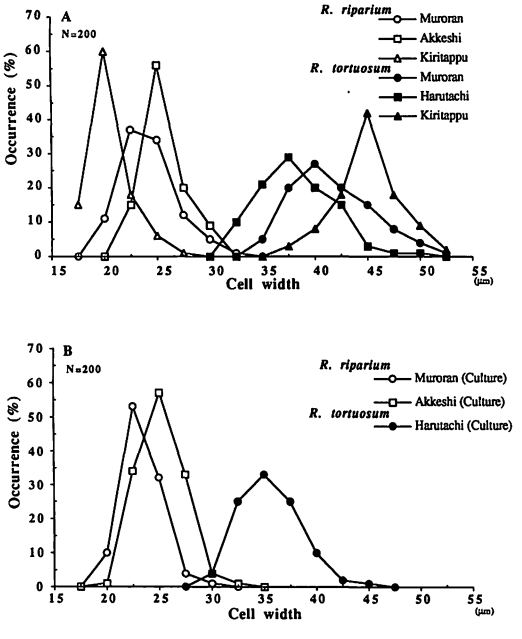

Fig. 1. Frequency curve of occurrence of cell width in two entities of *Rhizoclonium* collected from Hokkaido. A. Cell width in field plants of the two entities. B. Cell width in culture plants of the two entities.

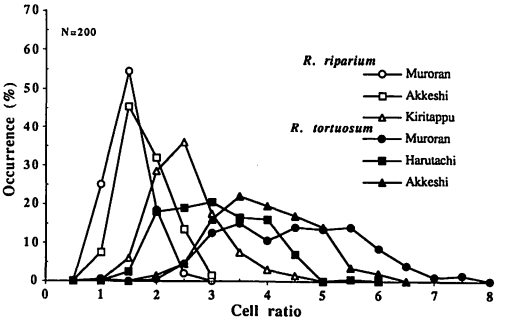

Fig. 2. Frequency curve of occurrence of length/width ratio of cells in field plants of two entities in *Rhizoclonium* collected from Hokkaido.

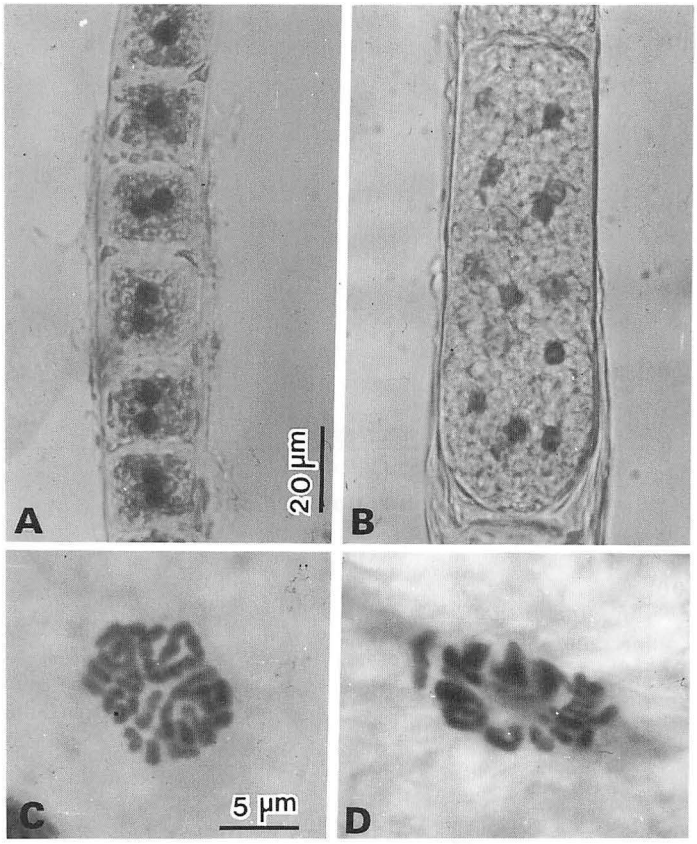

Fig. 3. *Rhizoclonium riparium* and *R. tortuosum* from culture. A. One or two nuclei in cells of *R. riparium* stained with aceto-carmine. B. Many nuclei in a cell of *R. tortuosum* stained with aceto-carmine. C. Chromosome of *R. riparium* showing a number of thirty-six. D. Chromosome of *R. tortuosum* showing a number of twenty four. Scale in A apply to B, and scale in C to D.

other algae were transferred to glass vessels (6.5 cm × 5 cm) containing 100 ml of ESP medium. The culture medium was replenished every 30 days or so. The cultures were kept in freezer-incubators illuminated with cool-white fluorescent lamps (*ca.* 4000 lux). Five temperature and photoperiod combinations were used: 5°C and 8 h of light, 10°C and 10 h or 14 h of light; 15°C and 14 h of light and 18°C and 16 h of light. The two entities grew well at 14 h of light; 15°C and 16 h of light, 18°C, but never became fertile for over a year at all conditions. For morphological observation of the two entities in culture, small clusters of vigorous plants were transferred to new vessel, placed at 15°C and 14 h of light, and incubated for one month. After this period, plants were fixed with 10% Formalin seawater. They were later used for the morphological observation and counting the nuclei. Morphology was similar to that of field plants. Intercalary rhizoidal branches in cultured plants of *R. riparium* were formed abundantly in the laboratory, as they are in the field. In cultured plants of *R. tortuosum*, intercalary rhizoidal branches were rarely formed, but this could not be observed in field plants. The frequency curves of occurrence of cell width and histograms of occurrence of the nuclear number of cultured plants in the two entities are similar to those of field plants (Fig. 1B, 4).

For observation of chromosomes, small clusters of vigorous cultured plants were transferred to a new vessel, placed at 15°C and 14 h of light, and incubated for three days.

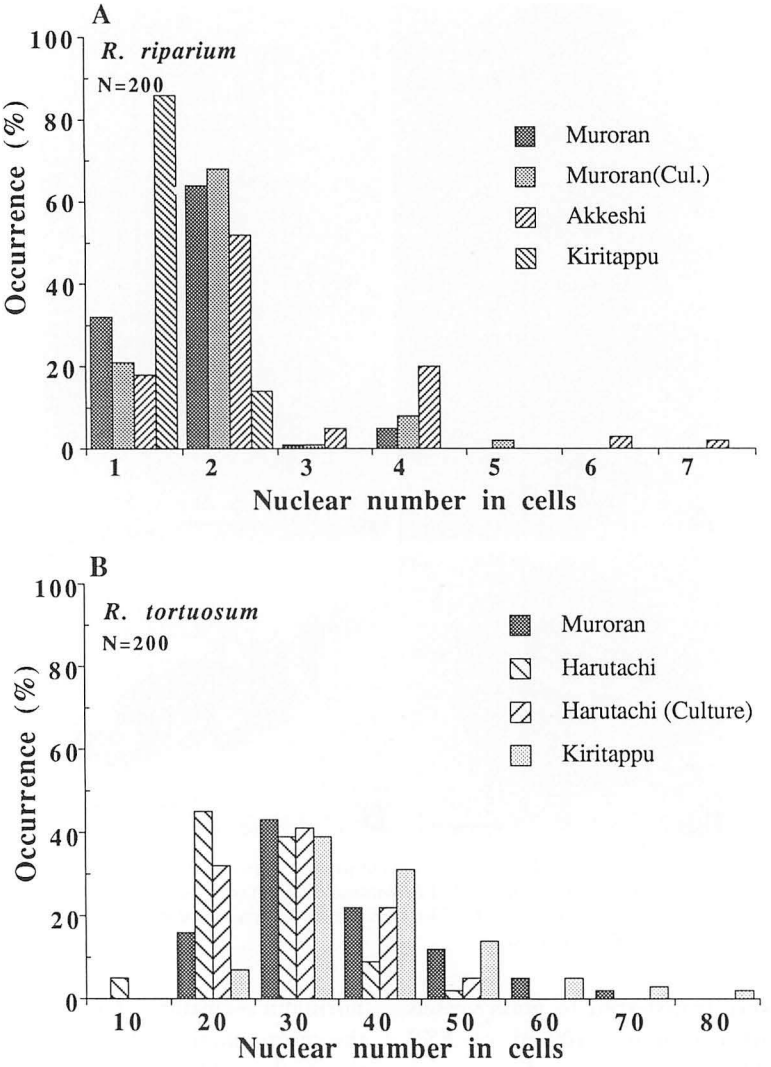

Fig. 4. Histogram of occurrence of nuclear number in a cell in *Rhizoclonium*. A. Nuclear number of *R. riparium*. B. Nuclear number of *R. tortuosum*.

Fixation was later performed with 3 : 1 ethanol: glacial acetic acid and, for the staining of chromosomes, aceto-iron-haematoxyl-in-chloral hydrate was used (Wittmann 1965). Ten chromosome counts per entity were made. At metaphase, (33-)36 chromosomes were counted in the vegetative cells of *R. riparium* (Fig. 3C). On the other hand, (22-)24 chromosomes were observed at metaphase in the vegetative cells of *R. tortuosum* (Fig. 3D). These chromosome numbers agree with the result of Sinha (1958). However, because the materials used did not reproduce, I could not be resolved whether the chromosome numbers of the two entities represent diploid or haploid numbers.

It is evident from this study that two species of *Rhizoclonium* are represented in Hokkaido, Koster (1955) has suggested that *Rhizoclonium riparium* and *R. tortuosum* are the same species, and the only difference between the two entities is cell width. However, nuclear number in a cell and the differences in chromosome number, in addition to cell width, clearly show that *Rhizoclonium riparium* and *R. tortuosum* from Japan should be regarded as two

distinct species (Fig. 2, 3C, 3D, 4). The two species are certainly separable on the bases of cell width. However, the differences of cell width in the two species between localities as shown Fig. 1A suggest that the cell width is not a good character. Instead, nuclear number in a cell and chromosome number should be adopted as a primary criterion for separating the two species. The ratio of cell width to length is not appropriate either as the frequency curves of the occurrence of various cell ratios between the two entities overlap (Fig. 2). The fact that the two entities occupy different, distinct habitats in any one place is further important evidence suggesting that the two separate species are involved.

The morphological characters and the habitat of Japanese *R. riparium* are essentially the same as those described by Koster (1955) and other authors. Migita (1967) first observed this species in Japan and described the habitat, phenology and morphology of this species in detail, and demonstrated the life cycle. I have obtained similar results except that it has not been possible to induce reproduction in culture.

Koster (1955) considered *Rhizoclonium tortuosum* to be a synonym of *R. riparium* f. *validum*, because of corresponding cell width in the two taxa. However, Foslie (1890), Rosenvinge (1893), Børgesen (1902) and Jónsson (1903) gave a different description of *R. riparium* f. *validum* and *Chaetomorpha tortuosa* (Dillwyn) Kützing; a synonym of *R. tortuosum*. In comparing of each description with Japanese plants, my observations on *R. tortuosum* does not agree with the former forma but, rather with the latter. My plants differ from *R. riparium* f. *validum* in habitat, nuclear number in cells and ratio of cell width to length. Foslie (1890), Jónsson (1903) and Waern (1952) found this entity in narrow supralittoral fissures of rock, and Koster (1955) recorded it from moist clayey or sandy soil. Jónsson (1903) noted two, or frequently, four nuclei in his *R. riparium* f. *validum*. With regard to ratio of cell width to length, Rosenvinge (1893), Jónsson (1903) and Koster (1955) described cells 1-2 times as long as broad. For these reasons, *R. tortuosum* and *R. riparium* f. *validum* may not be the same species.

Chaetomorpha tortuosa as described by Foslie (1890), Rosenvinge (1893), Børgesen (1902), Jónsson (1903), Kornmann (1972), and Kornmann and Sahling (1977) is similar to our observed plants in every respect. Rosenvinge (1893) and Kornmann (1972) noted about twenty nuclei in a cell in *C. tortuosa*. In this regard, Japanese plants agree completely with their *C. tortuosa*. Kützing placed *Conferva tortuosa* Dillwyn in *Chaetomorpha* at first (Kützing 1845) and later removed it to *Rhizoclonium* (Kützing 1849); Setchell and Gardner (1920) examined Kützing's original materials and concluded that *Ch. tortuosa* was synonymous with *Rhizoclonium tortuosum*, although they had never discussed whether this species should be regarded as belonging to the genus *Rhizoclonium* or *Chaetomorpha*.

Rhizoclonium tortuosum is also sometimes treated as a species of *Lola* (Hamel 1930; Chapman 1952, 1956); however, this genus has not been widely accepted. The question remains therefore: what is the correct genus name for Japanese plants known as "Naga-motsure" *Rhizoclonium, Chaetomorpha* or *Lola*? Until taxonomic confusion between the three genera is resolved with European material, I recommend *Rhizoclonium* as a generic name. Thus "Naga-motsure" is *Rhizoclonium tortuosum* (Dillwyn) Kützing.

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宮地和幸：北海道でのホソネダシグサとナガモツレ；両種の出現

ホソネダシグサ (*Rhizoclonium riparium* (Roth) Kützing ex Harvey) とナガモツレ (*R. tortuosum* (Dillwyn) Kützing) の両種は Koster (1955) らによって，同種のなかの品種として扱われたり，同種内の生態的変異型として扱われてきた。北海道ではホソネダシグサとナガモツレの両者が同じ場所に生育しており，同所産の標本で両者を比較することが出来る。両者が同一種なのか，それとも別種なのかを調べた。その結果，両者は細胞の幅だけでなく一細胞内の核数，染色体数，さらに生育潮位によって区別できた。ホソネダシグサは 17.5–35 μm の細胞の幅があり，一細胞内の核数は 1 - 8 個で，2 個が大半である。さらに，生育潮位は飛沫帯から潮間帯上部である。それに対して，ナガモツレは 30–60 μm の細胞の幅があり，一細胞内の核数は 8 個から80個までの変異があり，頻度の中心は20個から40個に存在する。さらに，生育潮位は潮間帯中部から潮間帯下部となっている。その他にも細胞の長さと幅の比にも若干の違いが見られ，ホソネダシグサは 0.7–4.5倍（多くは 1 –1.5倍），ナガモツレは 1.5–8.0倍（多くは 3 – 4 倍）である。これらの理由により，両者を同一種にして扱うよりは別種とし扱うほうが良いとの結論に達した。また，ナガモツレの学名はナガモツレを含めたこの周辺のグループの分類学的な問題が解決されるまでは *Rhizoclonium* 属に所属させる方が望ましい。(274 船橋市三山2-2-1　東邦大学理学部生物学教室)