Jpn. J. Phycol. (Sôrui) 39: 151–155, June 20, 1991

Sexual reproduction of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) in Japan

Dieter G. Müller* and Hiroshi Kawai**

*Fakultät für Biologie der Universität, D-7750 Konstanz, Fed. Rep. Germany
**Dept. of Botany, Faculty of Science, Hokkaido University, Sapporo, 060 Japan

Müller, D. G. and Kawai, H. 1991. Sexual reproduction of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) in Japan. Jpn. J. Phycol. 39: 151–155.

The cosmopolitan marine brown alga *Ectocarpus siliculosus* has been isolated from Hokkaido, Japan. The full sexual life history was obtained in clonal cultures: Gametophytes are dioecious and differ from sporophytes by a more elaborate branching pattern. Unfertilized gametes develop to partheno-sporophytes. Gametes of Japanese *E. siliculosus* are sexually compatible and form zygotes with isolates of the same species from various other geographic areas in both hemispheres. A revision of the genus *Ectocarpus* with presently over 40 species reported for Japan is recommended.

Key Index Words: cosmopolitan—ectocarpales—Ectocarpus siliculosus—*life history—Phaeophyceae—sexual reproduction.*

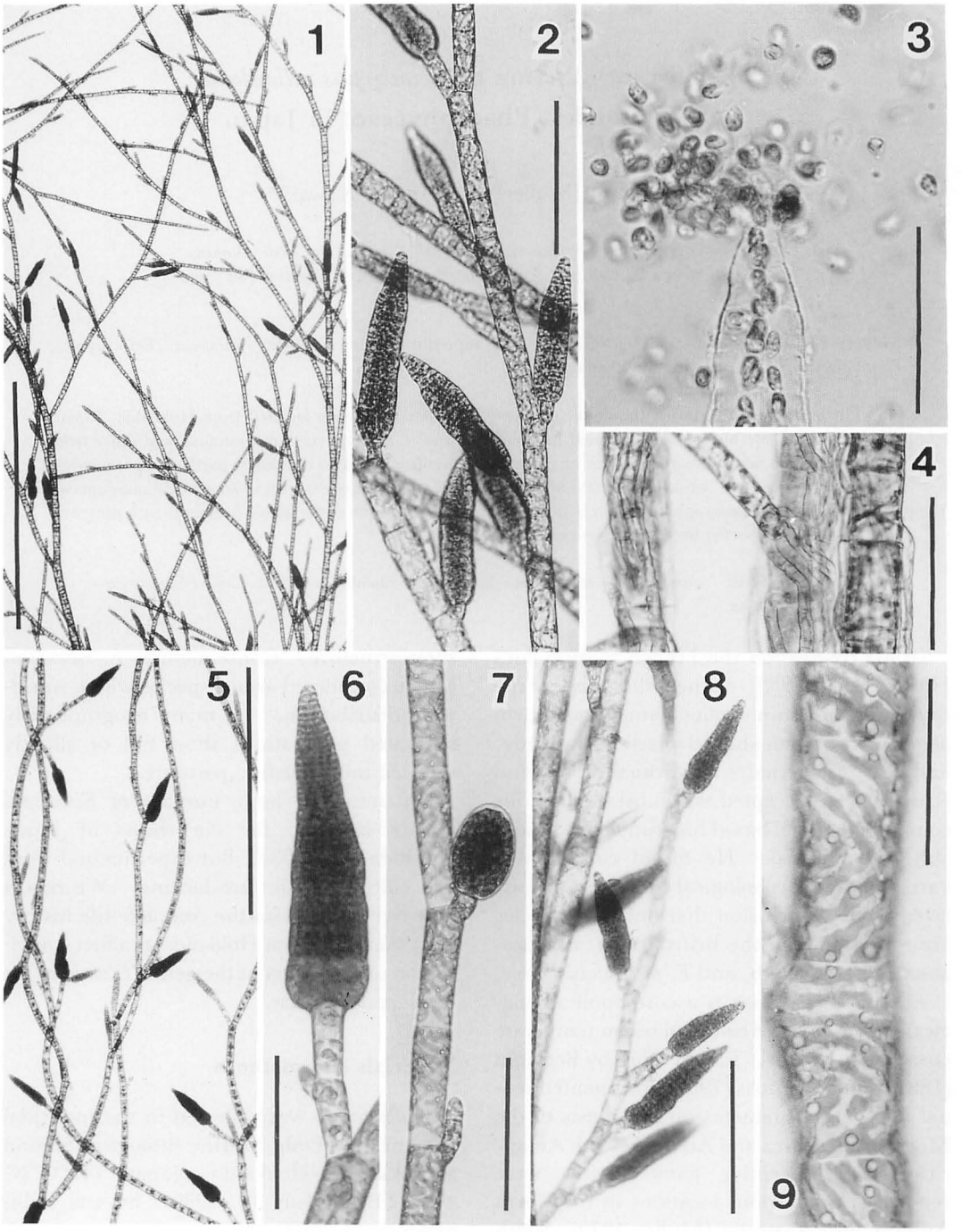
In his treatise on the French marine flora Hamel (1931-1939) defined the genus *Ectocarpus* to contain uniseriate filamentous brown algae with ribbon-shaped plastids, isogamy, and lack of true hairs. Cardinal (1964) and Russel (1966) accepted and substantiated this concept. Russell based his study on field and cultured material. He found considerable variability of morphological characters and arrived at the conclusion that only two species are represented at the British coast: *E. siliculosus* (Dillw.) Lyngb. and *E. fasciculatus* Harv.

Ectocarpus siliculosus is a cosmopolitan species, which inhabits cold and warm temperate coasts of all oceans. Earlier work by Berthold (1881) and Papenfuss (1935) documented sexual fusion of isogametes on the coasts of the Mediterranean Sea and Atlantic North America. More recently, gametophytes were reported from various locations in the North Atlantic and Australia (Müller 1979), South America (Müller 1988), and New Zealand (Müller *et al.* 1990). Crossing experiments showed that with few exceptions plasmogamy occurs between isolates from different geographic areas. In some cases segregation sterility indicates dissimilarities in chromosome structure. Consequently, *E. siliculosus* can be considered as one species with a worldwide distribution. Its many geographically separated populations show full or slightly reduced interbreeding patterns.

An unusually large number of *Ectocarpus* species is listed for the coasts of Japan (Yoshida *et al.* 1985), but experimental work and culture studies are lacking. We report here our findings on the complete life history of *E. siliculosus* from Hokkaido as a first contribution to a revision of the genus *Ectocarpus* for the Japanese coast.

Materials and methods

Leathesia sp. was collected in the intertidal zone near Akkeshi Marine Biological Station at Akkeshi, Hokkaido, Japan (43°02′N, 144°52′E) on July 18, 1989. Several millimeter-sized fragments were inoculated into plastic petri dishes in order to allow potential epiphytes to develop. By October 1989 an ectocarpoid filament appeared, which formed unilocular sporangia four weeks later. One unilocular sporangium was isolated. It released spores, which developed to gameto-



Figs. 1–9. Living cultured material of *Ectocarpus siliculosus* from Akkeshi, Hokkaido. 1: Habit of gametophyte, scale bar 1 mm. 2: Gametangia, scale bar 100 μm. 3: Gamete release from plurilocular gametangium, scale bar 50 μm. 4: Cortication in basal portion of older gametophyte, scale bar 100 μm. 5–7: Sporophyte, which developed from a zygote. Habit (Fig. 5); plurilocular sporangium (Fig. 6); unilocular sporangium (Fig. 7). Scale bars: 1 mm (Fig. 5); 100 μm (Figs. 6–7). 8: Partheno-sporophyte from unfertilized male gamete, scale bar 100 μm. 9: Gametophyte filament cells showing ribbon-shaped plastids with pyrenoids, scale bar 50 μm.

phytes. All work reported here was done with this material.

Unialgal cultures were maintained in enriched natural seawater (Provasoli-ES, Starr and Zeikus 1987) at 12±1°C. They were illuminated for 10 or 14 hr daily with a fluorescent lamp at an irradiance of 8–15 μmol m^{-2} s^{-1}. Experimental and culture techniques were the same as described previously (Müller 1988).

Specimens of our material will be deposited in the Herbarium of Department of Botany, Faculty of Science, Hokkaido University (SAP).

Results

The plants originating from the unilocular sporangium of an ectocarpoid epiphyte on a *Leathesia* species were male and female gametophytes of *Ectocarpus siliculosus* (Dillw.) Lyngb. This conclusion is based on the following morphological criteria: The plants are up to 5 cm long, and profusely branched in a sub-dichotomous manner without dominating main axis (Fig. 1). The plastids are ribbon-shaped with pyrenoids (Fig. 9), and male and female gametangia (Fig. 2) and gametes (Fig. 3) are isomorphic. True phaeophycean hairs are absent, and gametophytes are dioecious. Female isogametes settle on the substratum and attract male gametes until plasmogamy occurs. Zygotes develop to sporophytes of a few cm in size, which differ from the gametophytes by much sparser branching (Fig. 5) and larger plurilocular sporangia (Fig. 6). Older sporophytes and gametophytes show intense cortication by downward growing rhizoids (Fig. 4).

The spores from plurilocular sporangia develop to new sporophytes. Unilocular sporangia on the diploid zygotic sporophytes (Fig. 7) undergo meiosis, and their spores develop to a second generation of gametophytes with approximately equal representation of both sexes.

Unfertilized gametes develop parthenogenetically to plants with sporophyte habitus, which form unilocular and plurilocular sporangia (Fig. 8). These partheno-sporophytes were not studied further, since identical reproductive features have been found and studied in detail in *E. siliculosus* from Italy (Müller 1967).

In crossing experiments, gametes of our Japanese *Ectocarpus* cultures formed zygotes with gametes of *Ectocarpus siliculosus* from New Zealand (isolated by Müller *et al.* 1990), Naples, Italy, Wilmington, North Carolina (isolated by Müller 1979), and Chile (Müller 1988). Hybrids with the Italian isolates were fully viable, including functional meiosis. Hybrids with the North American and Chilean isolates showed reduced viability, while hybrid zygotes with New Zealand isolates did not develop (B. Stache. unpublished results).

Discussion

Morphological characters, life history, and sexual compatibility with isolates from different geographical areas provide convincing evidence that *Ectocarpus siliculosus* occurs on the coast of Japan. However, our study on cultured material does not indicate on which substrates and at what time of the year this species is expected to be found in the field in macroscopic scale. Specimens referable to *E. siliculosus* have not been collected at Akkeshi to date (Yamada and Tanaka 1944, Kawai, unpublished data).

The coasts of Japan belong to cold and warm temperate climatic zones. *E. siliculosus* is a typical representative for this temperature range, and in addition a cosmpolitan species. This opens the possibility that the genus concept for *Ectocarpus*, as specified by Russell (1966) for the British coast, may also be valid for Japan and other areas. An answer to this question requires detailed collection data as well as critical culture studies, which are not available at present.

The taxonomic treatment of the genus *Ectocarpus* in Japan is confusing. Yoshida *et al.* (1985) compiled all taxa reported for this area. The list includes *E. siliculosus* and *E. penicillatus* (C. Ag.) Kjellm., which is placed

by Hamel (1939) in the siliculosi group and considered by Russell (1966) as a transition form between *E. siliculosus* and *E. fasciculatus*. *E. breviarticulatus* J. Ag., which is also reported, has a tropical affiliation. The 44 additional taxa in the list are mainly new species, which were established by M. Noda and T. Ohta. According to their original diagnoses (Noda 1969, 1970, 1975, Ohta 1973), most of these taxa have discoid plastids, and consequently cannot be placed in the genus *Ectocarpus*. Instead, it seems likely that many of them belong to other genera such as *Feldmannia* or *Giffordia*, which has been recently renamed to *Hincksia* by Silva *et al.* (1987).

It is evident that a thorough reevaluation of type specimens, complemented with culture studies, will be needed to consolidate the taxonomy of the genus *Ectocarpus* in Japan.

Acknowledgements

We are grateful to Dr. T. Yoshida and the International Academic Exchange Program of Hokkaido University for a travel grant to D. G. M., and to the A. v. Humboldt Foundation (Bonn, FRG) for a research fellowship to H. K.

References

Berthold, G. 1881. Die geschlechtliche Fortpflanzung der eigentlichen Phaeosporeen. Mitt. zool. Stat. Neapel **2**: 401–413.

Cardinal, A. 1964. Étude sur les Ectocarpacées de la Manche. Beihefte Nova Hedwigia **15**, 86 pp.

Hamel, G. 1931–1939. Phéophycées de France. Paris. XLVII+432 pp.

Müller, D. G. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge *Ectocarpus siliculosus* in Kulturversuch. Planta **75**: 39–54.

Müller, D. G. 1979. Genetic affinity of *Ectocarpus siliculosus* (Dillw.) Lyngb. from the Mediterranean, North Atlantic and Australia. Phycologia **18**: 312–318.

Müller, D. G. 1988. Studies on sexual compatibility between *Ectocarpus siliculosus* (Phaeophyceae) from Chile and the Mediterranean Sea. Helgol. Meeresunters. **42**: 469–476.

Müller, D. G., Kawai, H., Stache, B. and Lanka, S. 1990. A virus infection in the marine brown alga *Ectocarpus siliculosus* (Phaeophyceae). Botanica Acta **103**: 72–82.

Noda, M. 1969. The species of Phaeophyta from Sado Island in the Japan Sea. Sci. Rep. Niigata Univ. ser. D. **6**: 1–64.

Noda, M. 1970. Some marine algae collected on the coast of Iwagasaki, Prov. Echigo facing the Japan Sea. Sci. Rep. Niigata Univ. ser. D. **7**: 27–35.

Noda, M. 1975. Some marine algae further added to the marine flora of Sado Island in the Japan Sea. pp. 91–107. In: Prof. Mitsuo Noda Commemorative Publication on his Retirement from the Faculty of Science. Niigata University, Niigata.

Ohta, T. 1973. Some new and rare marine algae from Tsugaru Straits between Honshu and Hokkaido. Sci. Rep. Niigata Univ. ser. D. **10**: 11–28.

Papenfuss, G. F. 1935. Alternation of generations in *Ectocarpus siliculosus*. Bot. Gaz. **96**: 421–446.

Russell, G. 1966. The genus *Ectocarpus* in Britain. I. The attached forms. J. Mar. biol. Ass. U. K. **46**: 267–249.

Silva, P. C., Meñez, E. G. and Moe, R. L. 1987. Catalog of the benthic marine algae of the Philippines. Smithonian Contr. Mar. Sci. No. **27**: 1–179.

Starr, R. C. and Zeikus, J. A. 1987. UTEX—The culture collection of algae at the University of Texas at Austin. J. Phycol. **23** Suppl.: 1–47.

Yamada, Y. and Tanaka, T. 1944. Marine algae in the vicinity of the Akkesi Marine Biological Station. Sci. Pap. Inst. Algol. Res., Fac. Sci., Hokkaido Univ. **3**: 47–77, pl. 8.

Yoshida, T., Nakajima, Y. and Nakata, Y. 1985. Preliminary checklist of marine benthic algae of Japan. I. Chlorophyceae and Phaeophyceae. Jap. J. Phycol. **33**: 57–74.

Müller, D. G.*・川井浩史**：日本産褐藻シオミドロ (*Ectocarpus siliculosus* (Dillw.) Lyngbye) の有性生殖

汎存種の褐藻シオミドロ（*Ectocarpus siliculosus*, シオミドロ目）を北海道，厚岸において採集し，クローン培養によって生活史と有性生殖について調べた。配偶体は雌雄異株であり，配偶体は胞子体より複雑な分岐をする点で形態上でも区別される。接合しなかった配偶子は雌雄のいずれも単為生殖の胞子体に発達する。日本産の *Ectocarpus siliculosus* は北半球および南半球の数地点から採集された本種の株と交配可能であることが確かめられた。日本においてこれまでに40種を越える *Ectocarpus* 属の種が記載されているが，本属の種で培養により有性生殖を含む生活史が明らかになったのはこの報告が初めてである。
(*Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, F.R.G.; **060 札幌市北区北10条西 8 丁目　北海道大学理学部植物学教室)