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The life history and evidence of the macroscopic male gametophyte in *Palmaria palmata* (Rhodophyta) from Muroran, Hokkaido, Japan

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Palmaria palmata was grown in cultures from spores and studied for cytology of fertilization process, life history and male gametophytic development. Tetraspores developed into female and male gametophytes in 1 : 1 segregation in cultures. The female gametophytes (discs) became mature by producing carpogonia with trichogynes even at 10～12 celled stage in 4～5-day-old culture. When the spermatia from locally collected plants were added to the female culture, they were readily attached to the trichogyne, the male nucleus entered into the trichogyne and fused with the carpogonium nucleus. The fertilized carpogonium formed a diploid erect thallus which consequently formed tetraspores in 6～7-month-old culture. The male gametophytes grew vegetatively and formed haploid erect thalli, on which the spermatia were formed in 3～4-month-old culture. Meiosis was observed in tetrasporangial first division. Morphological observations of macroscopic and microscopic plants of *P. palmata* from Muroran revealed some resemblances with *P. mollis* (S. & G.) VAN DER MEER and BIRD.

Key Index Words: female gametophyte—fertilization—life history—male gametophyte—Palmaria palmata—*Rhodophyta.*

The life history of *Palmaria palmata* (L.) KUNTZE has been studied by several workers (VAN DER MEER 1976, VAN DER MEER and CHEN 1979, VAN DER MEER and TODD 1980). YABU (1971, 1976) gave the cytological account on the chromosome numbers in this species (as *Rhodymenia palmata*). YABU and YASUI (1984), describing male gametophytic structure in the material (as *P. palmata*) from Hakodate, suggested that the male gametophyte also occurred in a microscopic form. However, the detailed cytological study of the fertilization process in *P. palmata* has not been done. In the present study we emphasized on the morphology and anatomy of male and female gametophytes and cytology of fertilization process. Also the diploid and haploid stages in the life history of *P. palmata* were confirmed. These results were similar to those demonstrated by VAN DER MEER and TODD (1980).

Elevation of *P. mollis* (S. & G.) VAN DER MEER and BIRD as an individual species from *P. palmata* f. *mollis* (S. & G.) GUIRY from the North Pacific Ocean and the comparison between these two species by VAN DER MEER and BIRD (1985) prompted us to reexamine the *P. palmata* from Muroran. *Palmaria* species collected along Muroran coast was so far referred as *P. palmata* (TAZAWA 1975, LEE 1978). In our observations, we found that this species showed more resemblance with *P. mollis* than with *P. palmata* from the North Atlantic Ocean.

Materials and Methods

Mature tetrasporophytes of *P. palmata* were collected from Muroran, Hokkaido. Tetraspores were cultured unialgally in PES medium (PROVASOLI 1966) at 10°C, and both 14 : $\overline{10}$ and 10 : $\overline{14}$ LD conditions under

55 μmol m^{-2} s^{-1} from cool-white fluorescent tubes. After the male and female gametophytes were differentiated from each other clearly (the female gametophyte is characterized by trichogynes), they were cultured separately. The spermatia obtained from locally collected mature male plants were inoculated on female gametophytes to observe fertilization process. For the cytological observations on fertilization and further developments, materials were fixed in 3 parts of 95% ethanol to 1 part of acetic acid for 2 hr and stained by aceto-iron-heamatoxylin method (WITTMANN 1965). To observe the fertilization process the slides were fixed at the intervals of 4, 6, 8, 10, 12 and 24 hr after the spermatium inoculation.

The anatomical details were compared with those given by GUIRY (1975), LEE (1978) and VAN DER MEER and BIRD (1985).

Results

Phenological and morphological observations

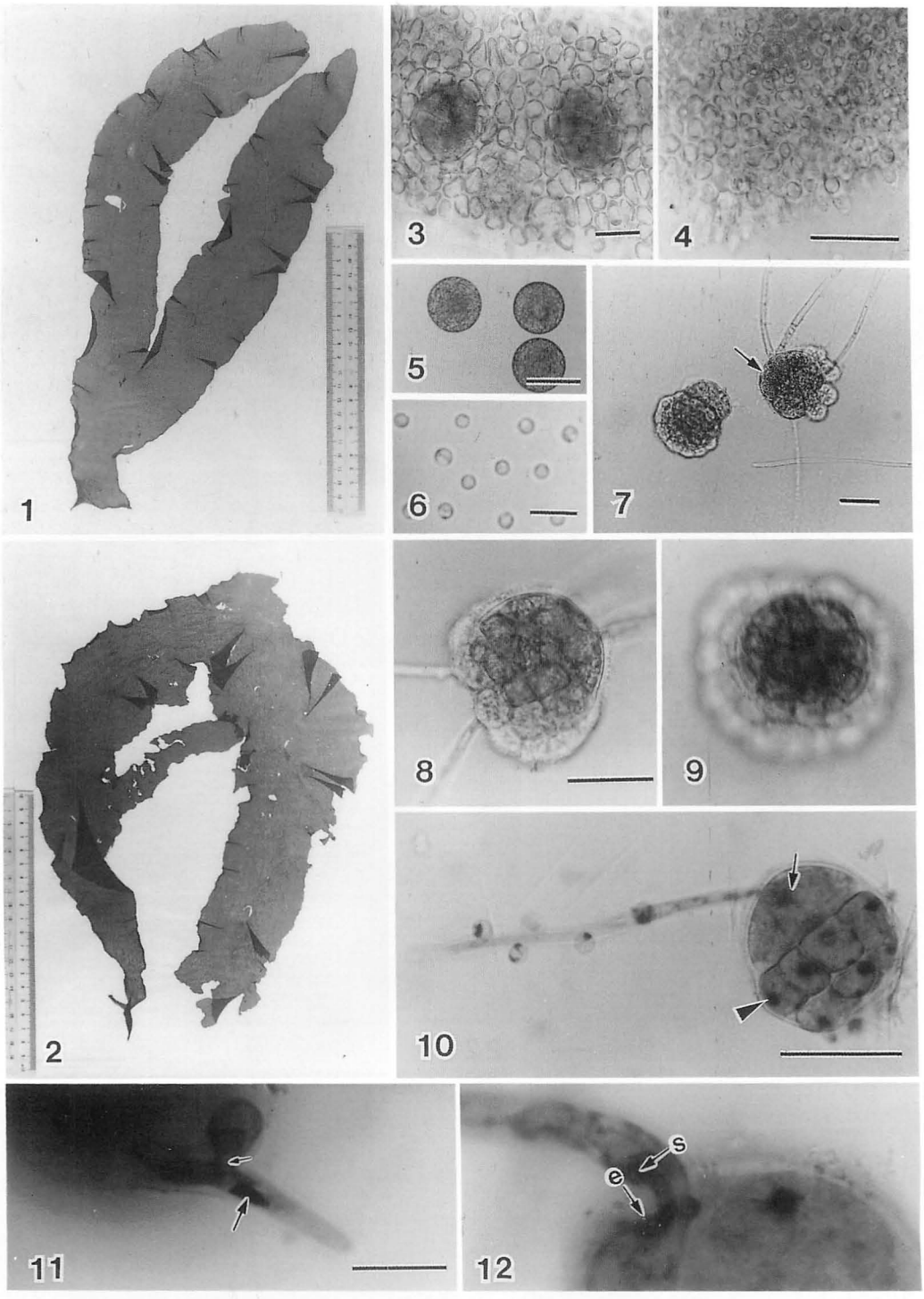
The mature thalli were found growing luxuriantly in number and size from December to May (upto 30-50~100 cm height) as described by LEE (1978). These thalli included both tetrasporophytes (Fig. 1) and spermatial thalli (Fig. 2), while some were sterile. The tetrasporic plants were large in number. The spermatial plants were also found frequently although a little less than tetrasporophytes.

The tetrasporic plants are easily distinguished by dark red in color from the pale colored spermatangial plants. The cortical cell size varied between 8-16 μm in diameter and the medullary cell size at the base of frond varied 130-410 μm in diameter. The arrangement of tetrasporangia confined to the outer cortical layer was found similar as showed by LEE (1978). The mature tetrasporangium was elliptical in transverse view and its size was 50-60 μm in length and 40-54 μm in width (Fig. 3). The mature spermatangia were oblong with size 8.6-11.2 μm in length and 3.4-4.8 μm in width (Fig. 4). The tetraspores were dark red in color and measured 15-20 μm in diameter, while the spermatia were light in color and 4.8-6.0 μm in diameter (Figs. 5, 6).

Culture experiments

The tetraspores germinated to form male and female gametophytes in 1 : 1 segregation (Fig. 7). Initial development of both the gametophytes was similar in form of prostrate disc. Within 4-day incubation, one cell of the female disc enlarged to form a carpogonium cell and a trichogyne emerged from the same cell. Sometimes more than one trichogyne emerging from the periphery were observed on the same disc (Figs. 7, 8). These carpogonium cells (eggs) were larger in size than the other disc cells and their nuclei were diffused, in contrast to the condensed vegetative cell nuclei. The female disc matured even at 10~12 celled stage (Fig. 10). Generally the disc of 100-125 μm size bears 18~50 trichogynes. These female discs, if not fertilized, grew into small erect

Fig. 1. Tetrasporophytic habit of *Palmaria palmata* from Muroran.
Fig. 2. *P. palmata* male gametophytic habit from Muroran.
Fig. 3. Surface view of tetrasporophytic thallus showing mature sporangia (scale=30 μm).
Fig. 4. Surface view of male gametophyte showing superficially arranged spermatangia (scale=30 μm).
Fig. 5. Released tetraspores (scale=20 μm). Fig. 6. Released spermatia (scale=15 μm).
Fig. 7. One: one segregation of male and female gametophytes after tetraspore germination in 4-day-old-culture (female is shown by arrow; scale=100 μm).
Fig. 8. Mature female gametophyte with 3 trichogynes (4-day-old) (scale=100 μm).
Fig. 9. Young male gametophyte with prostrate disc and centrally protruding erect thallus (scale=100 μm).
Fig. 10. Female gametophyte with an enlarged carpogonium having diffused nucleus (arrow) and vegetative cells with condensed nuclei (arrowhead). Five spermatia are seen attached to a single trichogyne (scale=30 μm).
Figs. 11, 12. Process of karyogamy (scale=10 μm). Fig. 11 showing migration of spermatium nucleus (large arrow) in the trichogyne after dissolving the trichogyne wall (small arrow). Fig. 12. Fusion between the spermatium and carpogonium nucleus takes place within 6-12 hr (s, spermatium nucleus; e, carpogonium nucleus).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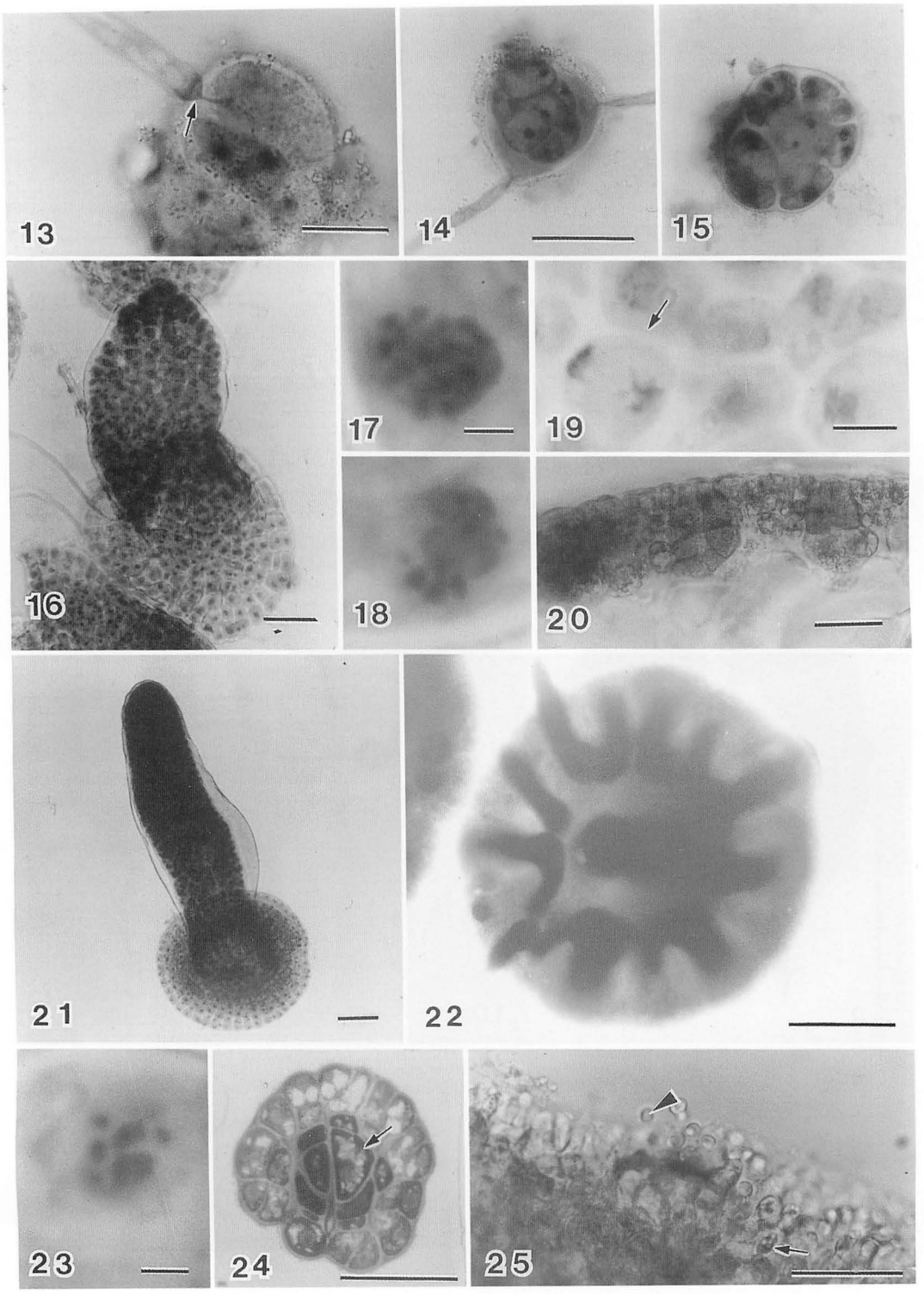
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thalli of 1 mm height with numerous long trichogynes and usually aborted in the culture dishes witin 2 months. The size of female disc varied from 100 to 500 μm in diameter.

Male discs showed a uniform growth and in 4～5-day-old incubation, their central cells divided obliquely to produce an erect thallus. The cells of erect thallus were easily distinguishable by dark red pigmentation (Fig. 9).

When adding the female cultures, the spermatia attached to the trichogyne within 4 hr. Attachment of more than one spermatium to a single trichogyne was quite a common feature (Fig. 10). After dissolving the trichogyne wall at the point of attachment, the spermatium nucleus migrated into the trichogyne and fused with the carpogonium nucleus (Figs. 11, 12). Once one spermatium nucleus fused with that of the carpogonium cell, the trichogyne of that cell became narrower forming a septum between itself and the carpogonium cell (Fig. 13). Thus the other spermatium nuclei which might have been entered in the same trichogyne could not enter in the carpogonium cell. The karyogamy took place within 6 to 12 hr. After fertilization, the carpogonium divided first transversely, and later vertically and obliquely, producing an erect thallus. No carpospore formation was observed. The erect frond developed directly on the female disc (Figs. 14-16). Growth of many erect thalli on a single female disc was due to one female disc bearing many carpogonia. These erect thalli were diploid where the disc still remained in haploid state. The diploid cells showed chromosome numbers 2n=40-42, and the haploid cells, n=20-21 (Figs. 16-18). Although the growth of these thalli was slower than the male plants, they developed into mature tetrasporic plants in 6～7-month-old culture (Figs. 19, 20).

The male gametophyte formed an erect thallus by protruding the central cells (Fig. 9). A 15～20-day-old erect thallus showed the uniform growth. Both the erect and disc cells were haploid (n=20-21) (Figs. 21, 23). In 1～2-month-old cultures, numerous male erect thalli showing the same chromosome numbers were observed growing radially on the same disc (Fig. 22). Figure 24 shows a cross section of young vegetative male disc. The light peripheral cells remained as the holdfast cells while the dark central cells dividing transversely and vertically formed the erect thallus. In 10°C and 14 : $\overline{10}$ LD culture condition the male plants matured within 3～4 months. In cross section (Fig. 25) the spermatangial mother cell was observed cutting off from the cortical cells. The spermatangial cluster was developed on these cells. The spermatangia were loosely arranged superficially on the cortex

Fig. 13. After the karyogamy, trichogyne of that carpogonium cell becomes narrow to form a septum (arrow) between the carpogonium cell and itself (scale=20 μm).

Figs. 14, 15. Development of fertilized carpogonium cells (scale=30 μm). Fig. 14 showing 2 celled and Fig. 15 showing 4 celled stage.

Fig. 16. Fertilized diploid erect thallus on the haploid disc. The disc persists the trichogynes (15～20-day-old) (scale=30 μm).

Fig. 17. Diploid nucleus from the erect thallus. 2n=40-42 (scale=2 μm).

Fig. 18. Haploid nuclues of the disc cell. n=20-21 (scale=2 μm).

Fig. 19. Tetraspore formation 1st division (arrow) in the tetrasporangium mother cell in 7-month-old culture (scale=5 μm).

Fig. 20. Cross section of tetrasporic thallus showing development of tatrasporangia in cortical region (scale=30 μm).

Fig. 21. Haploid male erect thallus from 15～20-day-old culture (scale=30 μm).

Fig. 22. Radial growth of many male thalli on the same disc (scale=400 μm).

Fig. 23. Male gametophytic cell with the haploid nucleus. n=20-21 (scale=2 μm).

Fig. 24. Cross section of young male gametophyte (4～5-day-old culture) showing vegetative stage. The central cells with dark pigmentation form the erect thallus (arrow) and the peripheral cells with light pigmentation remain as disc cells (scale=30 μm).

Fig. 25. Cross section of mature male plant (3～4-month-old culture) showing development of spermatangia. The spermatangial mother cells (arrow) cutting off from cortical cells from loosely arranged spermatangia on the thallus surface (arrowhead) (scale=30 μm).

and had the same morphology of those of male gametphytes from nature.

Discussion

The development of male and female plants in culture, the process of fertilization and the development of erect thallus of *P. palmata* from Muroran, Japan, show a similar pattern described for the order Palmariales (VAN DER MEER 1976, VAN DER MEER and CHEN 1979, VAN DER MEER and TODD 1980, VAN DER MEER and BIRD 1985). The chromosome numbers were similar to those observed by VAN DER MEER and CHEN (1979). Although there are many reports on the life history of the order Palmariales, very few reports give the detail picture of the fertilization process. MITMAN and PHINNEY (1985) studied fertilization and development of zygote in *Halosaccion americanum* using SEM. YABU and YASUI (1984) observed the migration of spermatium in the trichogyne of *P. palmata*. Both studies show the attachment and entry of the spermatia in the trichogyne. However, the actual karyogamic process was not described by these workers. The direct development of diploid erect thallus on the female disc has been very well demonstrated by VAN DER MEER and TODD (1980) by using green female for crossing; the diploid erect thallus, red in color, grew directly on the green female disc. In our experiment, we used both wild types and confirmed the diploid phase by chromosome counts.

The collections for anatomical features of our *Palmaria* plants showed the same characteristics given by TAZAWA (1975) and LEE (1978). The development of spermatia on the spermatangial mother cell was distinct in collected as well as cultured male plants. This is a common character of Florideae (TAZAWA, 1975). YABU and YASUI (1984) demonstrated particular development of male gametophytes. In their culture study, they described 1~8 celled mature male discs in 4-day-old culture; namely, the cell contents of such germlings divided rapidly into numerous minute granules to form spermatia. Further YABU and YASUI (1984) related existence of these dwarf male plants with the rare occurrence of macrophytic male gametophytes at Hakodate. However, during the present investigations we could not observe this kind of male gamete formation. HAWKES and SCAGEL (1986), discussing the life histories in Palmariales, expressed doubts on the existence of such a dwarf male until confirmation.

In our culture experiments, we observed that the development of male plant was not a rare phenomenon. In fact the male erect thalli were numerous. At our collection site near the Institute of Algological Research, Muroran (42°19′N; 140°59′E), we observed a frequent growth of male thalli. Previous reporters TAZAWA (1975) and LEE (1978) also noted the frequent growth of male *P. palmata* in Muroran area. However, in *P. marginicrassa*, LEE (1978) reported the rare occurrence of male plants in the field. Despite numerous growth of male plants in culture, they occur comparatively less in numbers in the field than the tetrasporophytes. The ecological reason for this situation is not known. GUIRY (1975) suggested that the plants were possibly neglected while making the collections. Hence a detail phenological study can help to understand the distribution of male and tetrasporophytic plants in the field.

TAZAWA (1975) and LEE (1978) studied the *P. palmata* from Muroran and retained at the same species level. Although LEE's (1978) anatomical description was comparable with those given by GUIRY (1975), he did not give any comparative account. In our culture experiments we observed many carpogonia producing numerous trichogynes toward the periphery of the disc. This result is quite similar to those illustrated by VAN DER MEER and BIRD (1985) and HAWKES and SCAGEL (1986). Therefore, on the basis of our observations we propose that the present species *Palmaria palmata* from Muroran should be regarded as *Palmaria mollis* (S. & G.) VAN DER MEER and BIRD. Also in this aspect specimens of *Palmaria* from other localities

along Japan coasts should be reexamined.

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DESHMUKHE, G. V.・舘脇正和：北海道室蘭産紅藻ダルスの生活史及び大形雄性配偶体

室蘭産紅藻ダルスの四分胞子の発芽体は培養において，1：1の割合で雌性及び雄性配偶体に生長した。雌性配偶体は4～5日培養で受精毛を伴った造果器を形成する。フィールドで採取した雄性配偶体の不動精子を培養の雌性配偶体に加えて受精させると，造果器は直ちに複相の直立葉を形成し，6～7ヶ月培養で成葉になり四分胞子を形成した。一方，雄性配偶体は栄養生長を続け単相の直立葉を形成し，3～4ヶ月培養で不動精子を形成した。また，フィールド及び培養藻体の形態観察から，室蘭産ダルスは *Palmaria mollis* (S. & G.) VAN DER MEER & BIRD との類似性が示された。(051 北海道室蘭市母恋南町1-13　北海道大学理学部附属海藻研究施設)