Journ. Fac. Sci., Hokkaido Univ., Ser. V (Botany), 13 (1): 25-38, 1983.

Morphology and life history in culture of a new variety of *Litosiphon groenlandicus* (Dictyosiphonales; Phaeophyta) from Japan

Hiroshi KAWAI and Munenao KUROGI

Litosiphon groenlandicus (Dictyosiphonales, Phaeophyta) known previously from the North Atlantic is reported with a new variety, var. *japonicus*, from Muroran, Hokkaido. It differs from the species in having a thicker thallus, larger medullary cells and producing only plurilocular sporangia. Swarmers of plurilocular sporangia develop into creeping filamentous microthalli provided with plurilocular sporangia. Swarmers of the plurilocular sporangia of microthalli develop again into the same microthalli. Under the long day conditions of 5°C, 10°C, 15°C and 20°C and the short day condition of 5°C, the microthalli develop the same erect macrothalli as seen in nature. The macrothalli produce again only plurilocular sporangia. Throughout the life history, sexual fusion of swarmers was not detected.

The genus *Litosiphon*, which was established by HARVEY (1849), had included two groups of species, one having hairs and the other not. Recently, PEDERSEN (1978) redefined the limits of this genus to include those species which have hairs with basal meristems. He segregated to *Pogotrichum* REINKE (1892) those species lacking hairs. In Japan, *Litosiphon* as so circumscribed has not been reported until now. Although *L. yezoense* YAMADA et NAKAMURA (1944), a species without hairs was earlier recognized, it has since (SAKAI and SAGA, 1981) been removed to *Pogotrichum*. An alga similar to *Litosiphon groenlandicus* with hairs was newly discovered in Muroran, Hokkaido. Morphological observations on the plant, the life history in culture, and the formation and fate of the swarmers from plurilocular sporangia are studied together with the taxonomy and are reported here. This is the second species of North Atlantic Dictyosiphonales recently reported for Japan, the first being *Delamarea attenuata* (KJELLMAN) ROSENVINGE by KAWAI and KUROGI (1980).

Materials and Methods

The materials were collected growing on stones together with young plants of *Scytosiphon lomentaria* (LYNGBYE) LINK in the upper subtidal zone

of approximately 1 meter in depth at Masuichi in Muroran (42°21'N, 140°59' E) on 31 May, 1980. A part of the materials collected was preserved in 10% formaldehyde-seawater for microscopic examinations.

For culture experiments, swarmers liberated from the plurilocular spotangia of the plant were pipetted on a glass slide. After their settlement, this slide was placed in a glass vessel containing 200 ml of PESI medium, a modification of PROVASOLI's ES medium (TATEWAKI 1966). The sets of culture conditions used were as follows: 16 : $\bar{8}$ at 5°C, 10°C, 15°C and 20°C and 8 : $\bar{1}\bar{6}$ at the same temperatures with the exception of 20°C. The illumination was 2000-3000 lx with fluorescent tube.

Result

Morphological observation

The plants are caespitose on stones, cylindrical, simple, solid and attenuated towards the base, yellowish brown in color, attaining 8 mm in height, 150-230 μm in diameter in the upper part (Fig. 4 a). The thallus is parenchymatous, composed of 1-2 layers of peripheral pigmented cells of 20-45 μm in diameter and 30-50 μm in height, and several large inner medullary cells of 63-93 × 45-88 μm in size in cross section (Figs. 1 a, b, 4 b). Peripheral cells are roundish or irregularly polygonal in surface view and somewhat protruding from the surface of thallus. Many discoid chloroplasts are contained in peripheral cells, but are poor to lacking in inner medullary cells. Hairs with basal meristems and sometimes with basal seathes are scattered over the entire surface of thallus. In the lower part of thallus, simple uniseriate rhizoidal filaments issue from the peripheral cells (Fig. 1 d). Only plurilocular sporangia are present in these plants. They are produced by the transformation of peripheral cells preceeded by vegetative divisions or not, somewhat protruding and irregularly conical in shape (Fig. 1 b, c, e). They are solitary or in sori, measuring 12-32 μm in diameter and 38-50 μm in height in cross section. The loculi measure 5-7 μm × 4-7 μm in length and width. Unilocular sporangia were not observed. In crude culture of the field materials at 5°C 16 : $\bar{8}$ LD for 2-3 weeks, the elongation of the plurilocular sporangia from the surface of the plant was observed (Fig. 1 f).

Culture experiments of swarmers

Swarmers liberated from the plurilocular sporangia are pear shaped, 7-8 μm × 4-5 μm in length and width, and provided with two lateral flagella, a chloroplast, a pyrenoid and an eyespot (Figs. 2 a, b, 4 c). They swim for a few minutes showing negative phototaxis and settle on the glass. Copula-

tion was not observed. After settlement, they become spherical (Figs. 2 c, 4 d). In 1-2 days, their chloroplasts increase in number and they elongate and germinate, dividing into two cells (Figs. 2 d, e, f, 4 e, f). By successive transverse divisions, they develop into prostrate uniseriate branched filaments (=microthallus) (Figs. 2 g, h, i, j, 4 g, h). Hairs were observed on the microthallus.

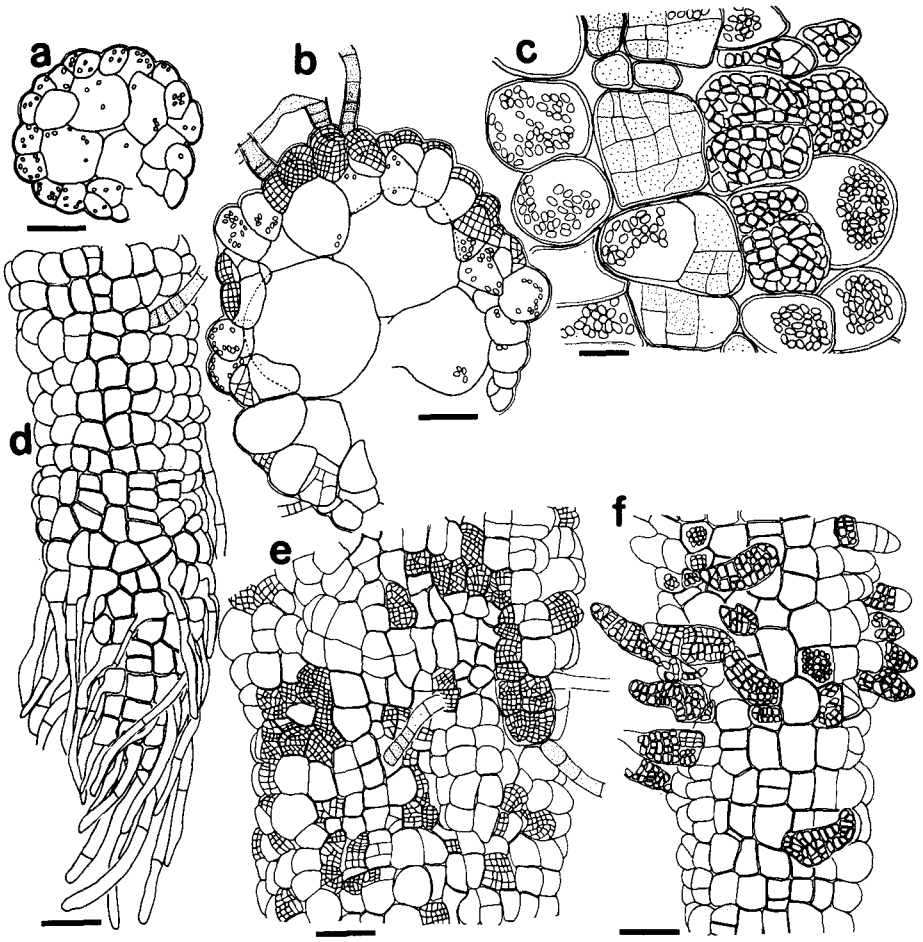

Fig. 1. *L. groenlandicus* var. *japonicus* in nature. a. Cross section of young erect thallus. b. Cross section of mature erect thallus with plurilocular sporangia and hairs. c, e. Peripheral cells, young and mature plurilocular sporangia in surface view. d. Basal part of erect thallus with rhizoidal filaments. f. Surface view of mature erect thallus after crude culture for 2-3 weeks, showing notably elongated plurilocular sporangia. Scale: (a, b, d-f)=50 μm, (c)=20 μm.

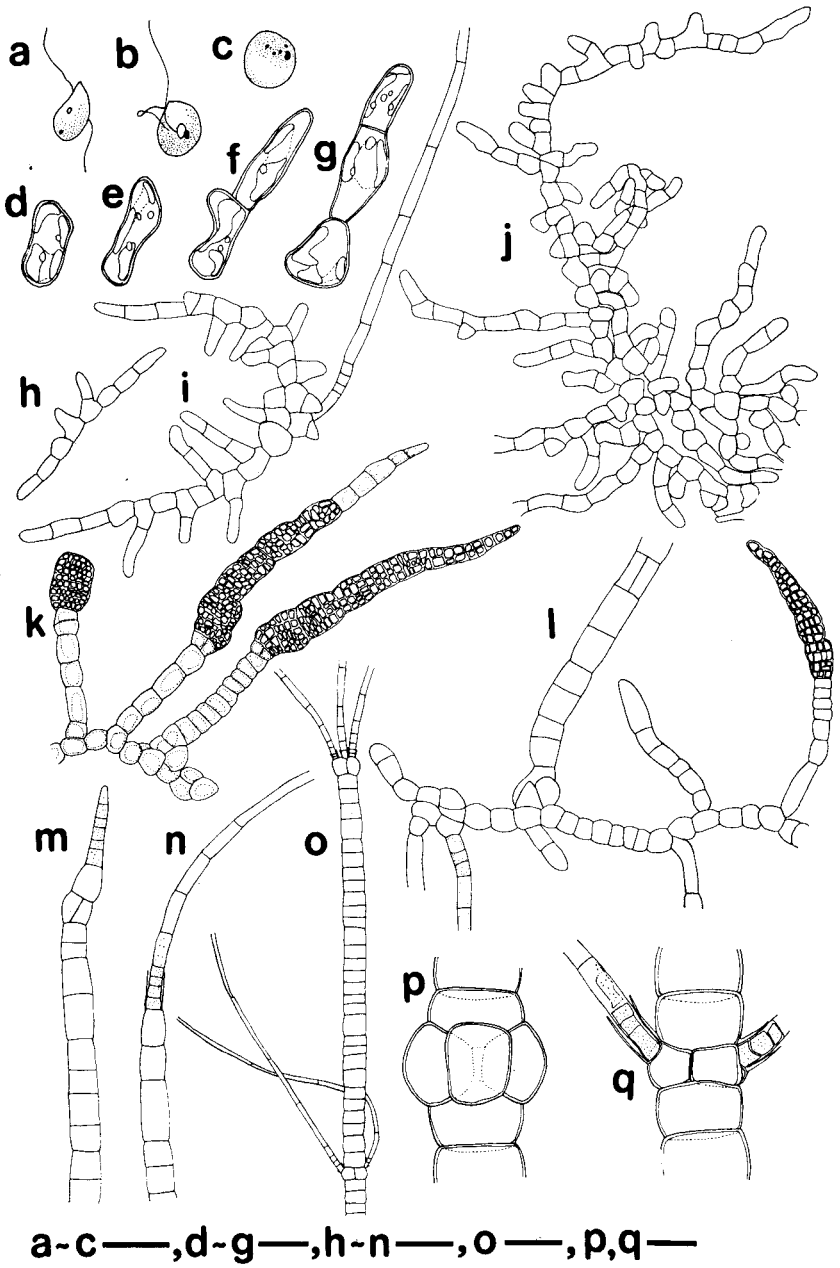

Fig. 2. *Litosiphon groenlandicus* var. *japonicus* in culture (1). a, b. Swarmers liberated from plurilocular sporangia of macrothallus. c. Settled swarmer. d-j. Germination of swarmer and development into microthallus with hairs. k. Plurilocular sporangia produced in microthallus. l. Young erect shoot issued from microthallus. m-o. Young erect shoots with terminal and lateral hairs. p, q. Young parenchymatous erect shoots. Scale: (a-g)=10 μm, (h-n)=50 μm, (o)=100 μm, (p, q)=200 μm.

In all culture conditions examined, intercalary or terminal long-lanceolate ectocarpoid plurilocular sporangia develop on the microthallus in 2-4 weeks (Figs. 2 k, l, 4 i). They are 110-193 μm in length and 15-30 μm in diameter, with 4-6 seriate loculi. The loculi measure 6-7 μm $\times$ 5-7 μm.

In the conditions of long day at 5°C, 10°C, 15°C, 20°C and short day at 5°C, initially uniseriate erect shoots (=macrothallus) showing intercalary growth with one to several terminal hairs are pushed out from the microthalli in 2-4 weeks (Figs. 2 l, m, n, o, 3 a, 4 j). Then they become parenchymatous by producing longitudinal cell walls (Figs. 2 p, 3 c, d). Opposite lateral hairs are often observed in young erect shoots (Figs. 2 q, 5 a). In the next stage of development, small peripheral cells are cut off and the thallus becomes macroscopic (Fig. 5 b). Within the temperature conditions examined, the growth of erect thallus is better at elevated temperature. Abnormal repeated branching is sometimes observed in higher temperature conditions, 15°C and 20°C (Fig. 5 c). Well developed erect thalli attain 40 mm in height and 350 μm ($-$450 μm) in diameter. In 4-6 weeks, conspicuous protruding plurilocular sporangia with 4-8 seriate loculi develop from the surface cells

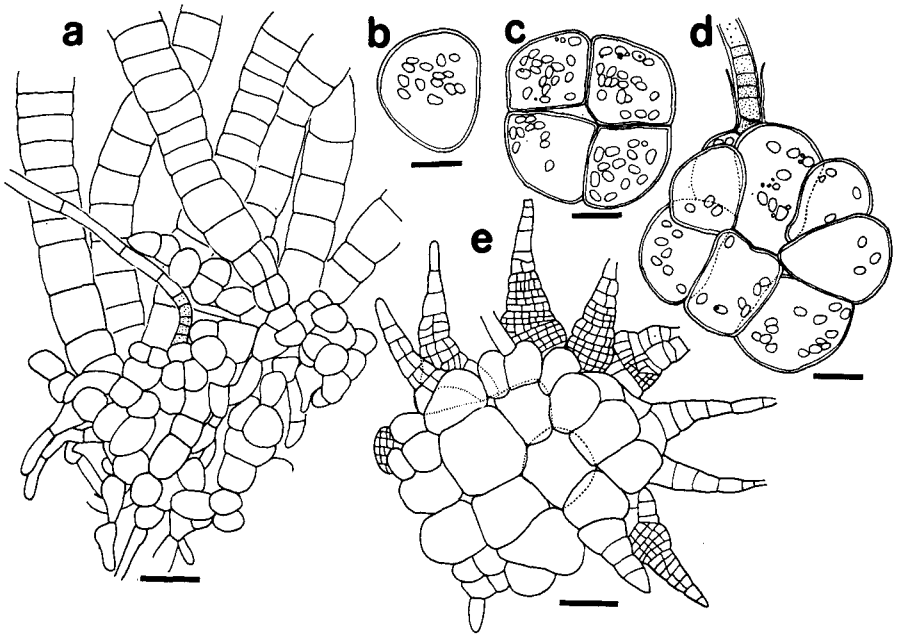

Fig. 3. *Litosiphon groenlandicus* var. *japonicus* in culture (2). a. Microthallus and young erect thalli. b-d. Development of erect thallus in cross section. e. Mature erect thallus with lanceolate plurilocular sporangia in cross section. Scale: (a, e)=50 μm, (b-d)=20 μm.

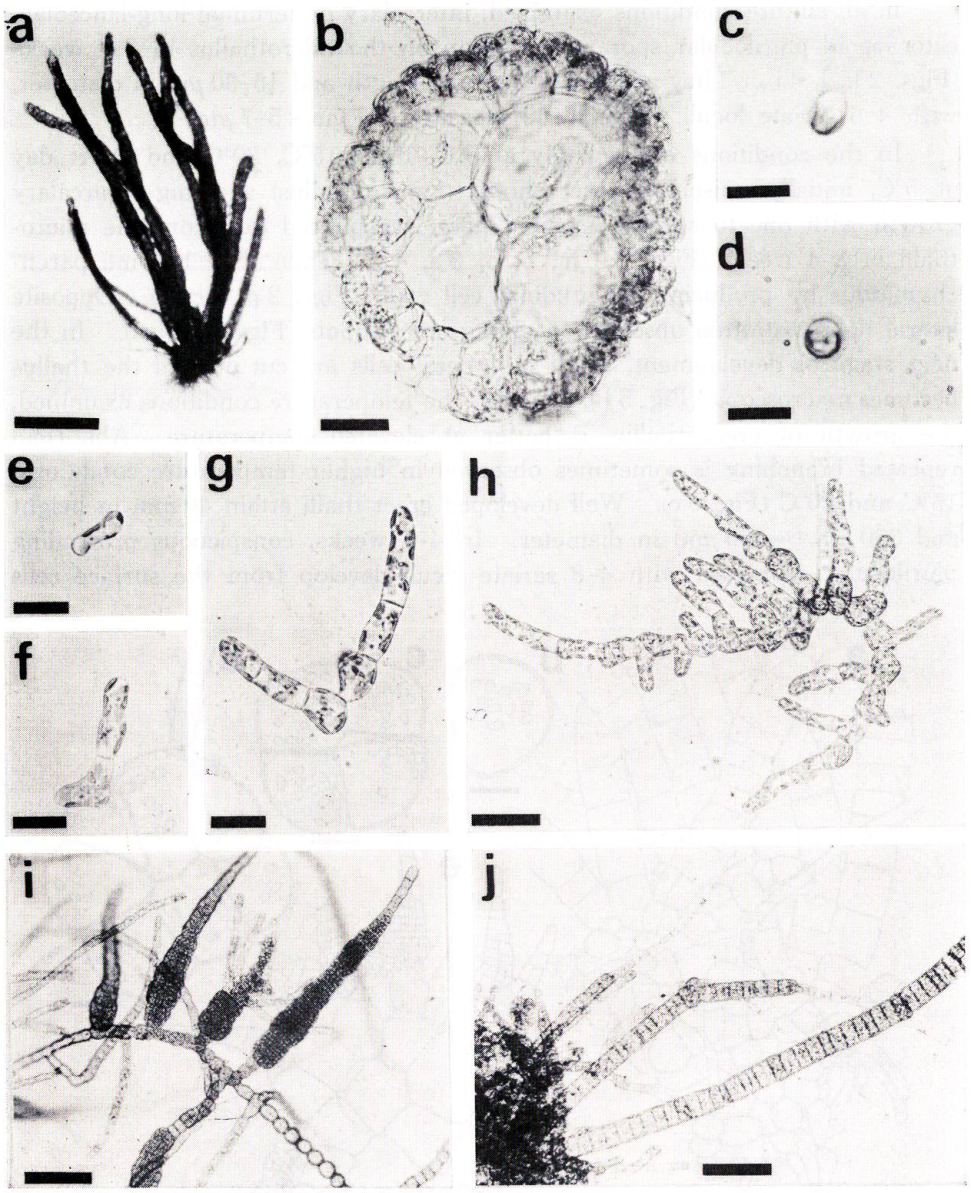

Fig. 4. *Litosiphon groenlandicus* var. *japonicus* in nature and in culture. a. Habit. b. Cross section of erect thallus from nature. c. swarmer liberated from plurilocular sporangium in macrothallus. d–h. Germination and development of the swarmer into microthallus. i. Plurilocular sporangia produced in microthallus. j. Young erect shoots pushed out from microthallus. Scale: (a)=2 mm, (b, h)=50 μm, (c, d)=10 μm, (e–g)=20 μm, (i, j)=100 μm.

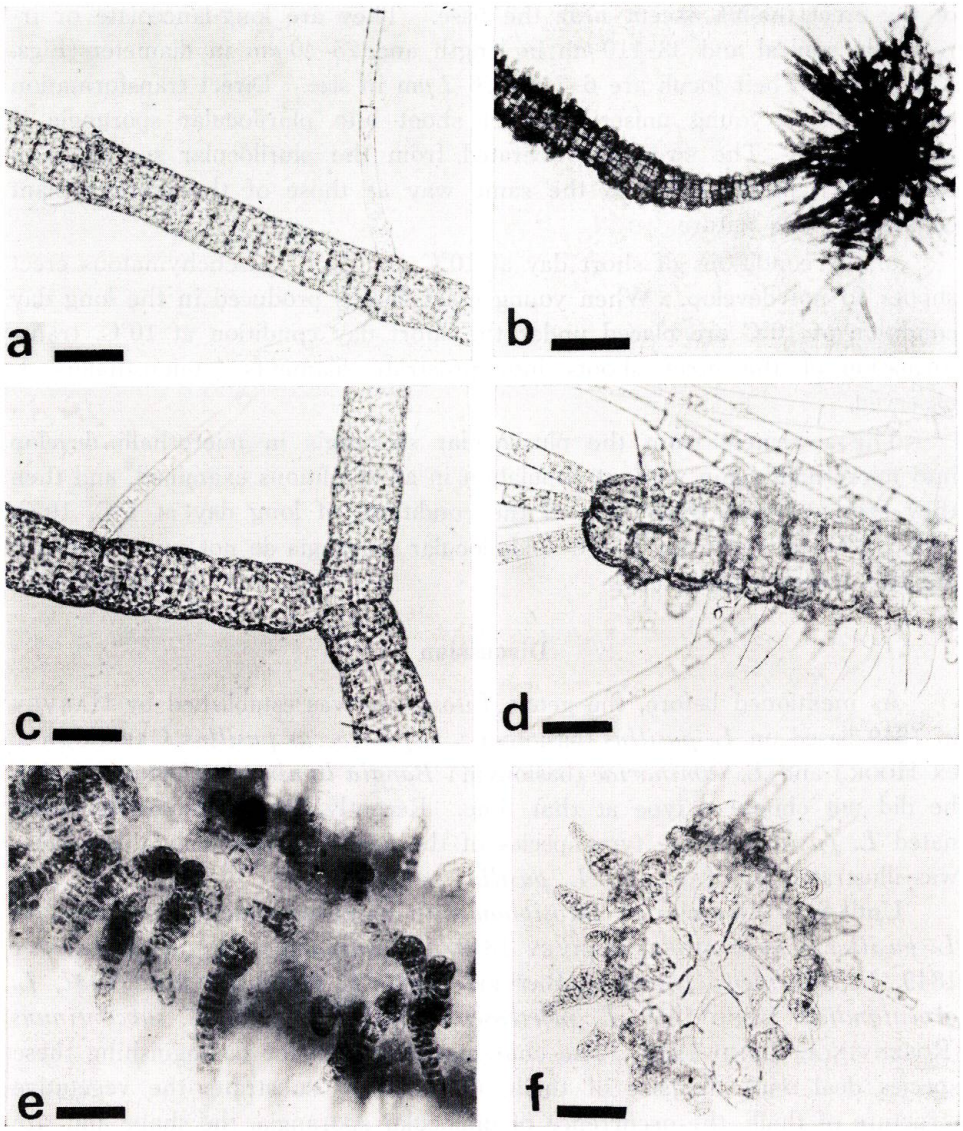

Fig. 5. *Litosiphon groenlandicus* var. *japonicus* in culture. a. Young erect thallus with lateral hairs. b. Microthallus with plurilocular sporangia and erect thallus. c. Rare occurrence of branching in erect thallus. d. Terminal part of erect thallus with hairs and empty plurilocular sporangia. e. Plurilocular sporangia on erect thallus. f. Mature erect thallus in cross section. Scale: (a, c–f)=50 μm, (b)=200 μm.

of the erect thallus except near the base. They are long-lanceolate or irregularly conical and 33–110 μm in length and 25–40 μm in diameter (Figs. 3 e, 5 e, f). Their loculi are 6–7 μm × 6–7 μm in size. Direct transformation of the cells in young uniseriate erect shoot into plurilocular sporangia is not observed. The swarmers liberated from the plurilocular sporangia in the erect thallus develop in the same way as those of the mother plant collected in the nature.

In the conditions of short day at 10°C and 15°C, parenchymatous erect shoots do not develop. When young erect shoots produced in the long day condition at 10°C are placed under the short day condition at 10°C, transformation of the erect shoots into prostrate filaments (=microthallus) is observed.

The swarmers from the plurilocular sporangia in microthalli develop into microthalli again without copulation in all conditions examined, and then they produce erect thalli only in the conditions of long day at 5°C, 10°C, 15°C, 20°C and short day at 5°C. Unilocular sporangia do not occur throughout the culture experiments.

Discussion

As mentioned before, the genus *Litosiphon* was established by HARVEY in 1849 based on *L. pusillus* (basionym : *Asperococcus pusillus* CARMICHAEL ex HOOK.) and *L. laminariae* (basionym : *Bangia laminariae* LYNGBYE), but he did not choose a type at that time. Recently, PEDERSEN (1978) designated *L. pusillus* as the type species of the genus because only this species was illustrated by HARVEY. *L. pusillus* has hairs.

Until now 7 species of *Litosiphon* with hairs are described. They are *L. pusillus* (CARMICHAEL) HARVEY 1849, *L. laminariae* (LYNGBYE) HARVEY 1849, *L. hibernicus* (JOHNSON) BATTERS 1902, *L. tenuis* LEVRING 1937, *L. groenlandicus* LUND 1959, *L. mortensenii* LUND 1959, and *L. subcontinuus* (ROSENVINGE) LUND 1959. The characteristics used for distinguishing these species deal with the size of thalli, the kind of substrate, the vegetative structure of thalli, the occurrence of unilocular sporangia, the shape and size of plurilocular sporangia, and the size of loculi in the plurilocular sporangia. Among these characteristics, the occurrence of unilocular sporangia does not seem to be so significant. In the type species, *L. pusillus*, a seasonal alternation between plurilocular sporangia and unilocular ones is reported by SAUVAGEAU (1929) and KYLIN (1933). To compare our plant in nature with them, some of their characteristics are shown in Table 1. Our plant differs from most of the species except *L. groenlandicus* and *L. subcontinuus* in having

TABLE 1. Comparison of main features of the seven species and a new variety of *Litosiphon* with hairs

Species \ Features	Height & diameter of thalli	Substrate	Structure of medulla of thalli	Reproductive organ	Origin and shape of pluril. sporangia, and size of loculi
L. pusillus (HARVEY 1849)	ca. 5–15 cm —	epiphytic	3–5 layers of ± isodiametric cells (from illustration)	—	—
L. pusillus (ROSENVINGE & LUND 1947)	3–6 cm 90–175 μm	epiphytic	—	unil.+pluril.	transformed from peripheral cells,
L. pusillus (HAMEL 1937)	1–10 cm 250–1000 μm	epiphytic	several layers of ± isodiamertic cells	pluril.+unil.	transformed from peripheral cells,
L. laminariae (HARVEY 1849)	ca. 0.6–1.3 cm —	epiphytic	—	—	—
L. tenuis (LEVRING 1937)	−1 cm —	epiphytic	3–4 layers of cells	unil.	—
L. hibernicus (JOHNSON 1894) as *P. hibernicus*	−1 cm —	rhizoidal base endophytic	several layers of ± isodiametric cells	pluril.	—
L. groenlandicus (LUND 1959)	−4 cm 50–200 μm	epiphytic & epilithic	single layer of large cells	pluril.+unil.	transformed from peripheral cells, irregular conical, 5–7×4–5 μm
L. groenlandicus var. *japonicus* in nature	−8 mm 175–400 μm	epilithic	single layer of large cells	pluril.	transformed from peripheral cells, irregular conical, 5–7×4–7 μm
L. mortensenii (LUND 1959)	−2 cm −125 μm	epiphytic	—	pluril.	transformed from peripheral cells, irregular conical, 7–10×7–8.5 μm
L. subcontinuus (LUND 1959)	−4 cm −150 μm	epiphytic & epilithic	single layer of large cells	pluril.	issued outward from peripheral cells, cylinerical,

a single layer of large medullary cells in cross section. Our plant agrees with *L. groenlandicus* in the kind of substrate, in the vegetative structure of thallus, in the shape of plurilocular sporangia and in the size of loculi of the sporangia, but differs in the height and diameter of thalli. *L. subcontinuus* is different from our plant in having characteristic uniseriate plurilocular sporangia, a morphological feature of some stability. Table 2 shows a comparison of our plants in nature and in culture (well developed erect thalli in long day conditions) with *L. groenlandicus* as reported in detail by LUND (1959). It is found that the diameter of thalli and size of medullary

TABLE 2. Comparison in morphology of *Litosiphon groenlandicus* var. *japonicus* in nature and in culture with *L. groenlandicus*

	our plant in nature	our plant in culture	*L. groenlandicus* in LUND 1959
height of thallus (mm)	−8	−40	−40
diameter of thallus (μm)	175–400	190–350 (−450)	50–200
length×width of peripheral cells (μm)	30–50×20–50	28–55×24–43	14–40×12–32
length×width of inner cells (μm)	63–93×45–88	80–130×80–120	−50 (from illustration)
diameter of hairs (μm)	13–18	9–13	8–16
plurilocular sporangia shape	somewhat protruding, irregular conical	protruding, lanceolate	somewhat protruding, irregular conical
length×width (μm)	38–50×13–32	33–110×25–40	16–32×14–32
size of loculi (μm)	5–7×4–7	6–7×6–7	5–7×4–7
unilocular sporangia	unknown	unknown	known

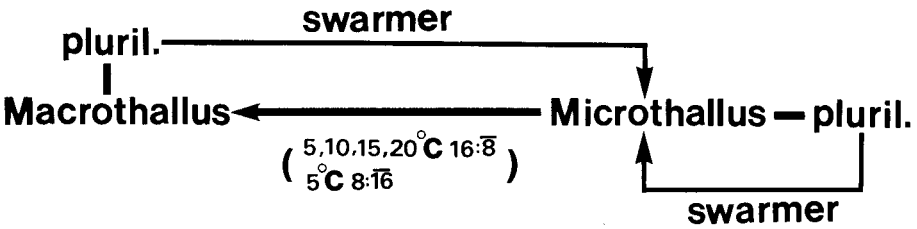

Fig. 6. A diagram of life history in culture of *Litosiphon groenlandicus* var. *japonicus*.

cells of our plant in nature and in culture are larger than those of *L. groenlandicus*. It is difficult to conclude whether the difference is ecological or genetical. Taking into account all of our observations, we regard the difference between our plant and *L. groenlandicus* as that at infraspecific level and conclude by treating our plant as a variety of *L. groenlandicus*.

A diagram of the life history of our plant in culture is shown in Fig. 6. The plant (=macrothallus) produces only plurilocular sporangia. The swarmers develop into prostrate branched uniseriate filaments (=microthallus) producing lanceolate plurilocular sporangia. The swarmers from microthalli develop into the same kind of microthalli with plurilocular sporangia again. Only in the conditions of long day at 5°C, 10°C, 15°C and 20°C, and of short day at 5°C, the microthalli produce erect macrothalli on them together with plurilocular sporangia. In the conditions of short day at 10°C and 15°C erect macrothalli do not develop from the microthalli. Unilocular sporangia are not found on macrothalli nor on microthalli. No copulation is detected in the swarmers of macrothalli and in those of microthalli. From these results, there seems to be no alternation of generations followed by alternation of nuclear phases in this plant.

As to the life histories of the related species, KYLIN (1933) studied on *Litosiphon pusillus* which produces plurilocular sporangia in summer and unilocular sporangia in autumn. He showed the culture experiments that swarmers from plurilocular sporangia developed into the same erect macrothalli as seen in nature and swarmers from unilocular sporangia into creeping filamentous microthalli with uniseriate plurilocular sporangia. He regarded the creeping microthalli as gametophytes and postulated the heteromorphic alternation of generation in *L. pusillus*, in spite of no evidence of copulation of gametes. SAUVAGEAU (1929, 1933) and NYGREN (1975) obtained also in culture the microthalli with plurilocular sporangia as described by KYLIN. However, they reported the development of erect filaments (short macrothalli) on the microthalli and no sexual fusion of swarmers of plurilocular sporangia of microthalli.

Nearly the same life history without sexual generation is known in *Pogotrichum filiforme* (PEDERSEN 1978) and *P. yezoense* (SAKAI and SAGA 1981). *P. filiforme* produces only plurilocular sporangia and *P. yezoense* both plurilocular and unilocular sporangia. Swarmers of both the sporangia develop into creeping filamentous microthalli with plurilocular sporangia, on which erect macrothalli develop under certain temperature and photoperiod conditions. Copulation of swarmers of the plurilocular sporangia is not observed in either of both the species.

Diagnosis

Litosiphon groenlandicus* var. *japonicus KAWAI et KUROGI var. nov.

Plantae filiformes, ad 8 mm longae, 175-400 μm diametro; cellulae externae thalli 30-50 μm longae, 20-45 μm latae; cellulae medullae multo majores 63-93 $\times$ 45-88 μm magnitudine; pili 13-18 μm crassi; sporangia plurilocularia plerumque prominentia, obtuse irregulariter conica, 38-50 μm longa, 13-32 μm diametro, multiseriata, loculis 5-7 $\times$ 4-7 μm magnitudine; sporangia unilocularia ignota.

Holotypus: Plurilocular sporangial, 31 May 1980, Masuichi in Muroran, Hokkaido, collected by Hiroshi KAWAI (SAP 035665).

Plant filiform, simple, up to 8 mm (40 mm in culture) in length, 175-400 μm in diameter; thallus composed of 1-2 layers of peripheral cells 30-50 μm in length and 20-45 μm in diameter and several large medullary cells 63-93 $\times$ 45-88 μm in size; hairs present, 13-18 μm in diameter, with multiseriate loculi of 5-7 $\times$ 4-7 μm in size; unilocular sporangia unknown.

We wish to acknowledge Professor Isabella A. ABBOTT, Stanford University, for her critical reading, and Professor Yoshio SAKAI, Institute of Algological Research, Hokkaido University, for his help in collecting the sample in Muroran.

References

HAMEL, C. 1937. Phéophycées de France. Paris.

HARVEY, W. H. 1849. A manual of the British marine algae. 2nd ed. London.

JOHNSON, T. 1894. Two Irish brown algae: *Pogotrichum* and *Litosiphon*. Ann. Bot. **8** (32): 457-464, pl. 24.

KAWAI, H. and KUROGI, M. 1980. Morphological observation on a brown alga, *Delamarea attenuata* (KJELLMAN) ROSENVINGE (Dictyosiphonales) new to Japan. Jap. J. Phycol. **28** (4): 225-231.

KYLIN, H. 1933. Über die Entwicklungsgeschichte der Phaeophyceen. Lunds Univ. Årsskr., N. F. Avd. 2. **29** (7): 5-102, pls. 1-2.

LEVRING, T. 1937. Zur Kenntnis der Algenflora der norwegischen Westküste. Lunds Univ. Årsskr. N. F. Avd. 2. **33** (8): 5-147, pls. 1-4.

LUND, S. 1959. The marine algae of East Greenland 1. Taxonomical part. Medd. Grønland. **156** (1): 1-247.

NYGREN, S. 1975. Life history of some Phaeophyceae from Sweden. Bot. Mar. **18**: 131-141.

PEDERSEN, P. M. 1978. Culture studies on marine algae from West Greenland III. The life histories and systematic positions of *Pogotrichum filiforme* and *Leptonematella fasciculata*. Phycologia **17** (1): 61-68.

REINKE, J. 1892. Atlas deutscher Meeresalgen II. Berlin, pp. 35-70, pls. 26-50.

SAKAKI, Y. and SAGA, N. 1981. The life cycle of *Pogotrichum yezoense* (Dictyosiphonales, Phaeophyceae). Sci. Pap. Inst. Algol. Res., Fac. Sci., Hokkaido University, **7** (1): 1–15, pls. 1–6.

SAUVAGEAU, C. 1929. Sur le developpement de quelques Phéosporées. Bull. Stat. biol. Arcachon **26**: 253–420.

SAUVAGEAU, C. 1933. Sur quelques algues Phéosporées de Guéthary. Ibid. **30**: 1–128.

TATEWAKI, M. 1966. Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. Phycologia **6**: 62–66.

YAMADA, Y. 1944. Notes on some Japanese algae X. Sci. Pap. Inst. Algol. Res., Fac. Sci., Hokkaido Imp. Univ. **3**: 11–25.