

## CHROMOSOME STUDY ON THE SUBMERGED MOSS COLLECTED FROM ANTARCTIC LAKES

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**Abstract:** The submerged moss, which was found on lake beds in the Syowa Station area, East Antarctica was studied karyologically and taxonomically. The chromosome number of the moss showed to have  $n=23$  and is expressed as  $n=22+m$  because it has a smallest chromosome with negative heteropycnosis. It may be appropriate to assign this moss to *Leptobryum pyriforme* (HEDW.) WILS. judging from the chromosome number in addition to some morphological features such as sexuality and tubers.

### 1. Introduction

A karyological study is significant in determining taxonomical position of mosses and it seems to be effective for analyzing Antarctic mosses which are usually sterile. Three karyological studies have so far been carried out for the materials from the Syowa Station area (TATUNO, 1963; INOUE, 1976; KANDA and OKADA, 1990), but anyone has never studied chromosomes of submerged mosses known in the area.

Some submerged mosses are known from lakes of the Syowa Station area, Enderby Land, Antarctica (NAKANISHI, 1977; KANDA and IWATSUKI, 1989; KANDA and OHTANI, 1991; KANDA and MOCHIDA, 1992). Based on the submerged moss specimens collected by NAKANISHI (1977), IMURA and KANDA (1986) described the morphology of globose gemmae, which occurred on smooth rhizoidal stalks. They suggested that this moss seems not to belong to Bryaceae judging from tubers with short, smooth lateral rhizoids and cell shapes of leaves. KANDA and IWATSUKI (1989) revised this moss taxonomically and tentatively referred it to a species of *Dicranella*, whose morphological features were closely similar to those of *D. palustris* (DICKS.) CRUNDW. ex WARB. occurring in the temperate regions. However, the decision of the taxonomic position was deferred to the further study, because most of the morphological features vary when the plant is submerged.

When joined the 29th Japanese Antarctic Research Expedition (JARE-29, 1987–89) for a botanical study as one of the overwintering members, the senior author collected a submerged moss ("*Dicranella* sp.") occurring on lake beds near Syowa Station. It was the second collection since NAKANISHI first collected this moss in 1975 (NAKANISHI, 1977). In the present article, the chromosome number of this submerged moss is reported and taxonomical position of the specimen is discussed.

## 2. Material and Methods

The moss specimen (No. 890620-352) was collected from lake bed of the ice-free area, Byvågåsane near Syowa Station on the 11th October 1988. It was frozen and stored at low temperatures of approximately  $-20^{\circ}\text{C}$  within ten days after collection. After six months, the specimen was thawed and used for culture. Plants which had been grown at  $10^{\circ}\text{C}$  for about three years in BBM culture were incubated again on agar culture medium (0.05% Hyponex (Hyponex Co.), 1.5% agar) at  $14^{\circ}\text{C}$  and new shoots were brought out. Chromosome observations were carried out by the same methods as the previous report (KANDA and OKADA, 1990). Well-grown new shoots were pre-treated with 0.05% colchicine aqueous solution at  $18^{\circ}\text{C}$  for 4 hr. Air among tissues was removed using a vacuum pump. The shoots were fixed with fresh modified Calnoy's fluid (ethanol:chloroform:acetic acid=2:1:1) at  $5^{\circ}\text{C}$  for longer than 1 hr. They were rinsed with distilled water. The cell walls were digested by an enzyme mixture (2% cellulase, Onozuka Yakult Co., RS, 0.2% pectolyase, Seishin Co., and 0.6 mol mannitol, pH 5.5) at  $37^{\circ}\text{C}$  for 1.5 hr. The shoots were then rinsed with distilled water again and the meristematic tissues were put on a slide glass and stained with 2% aceto-orcein for 30 min. They were covered with a coverslip and warmed up to  $60^{\circ}\text{C}$  for several seconds and squashed.

The moss specimen studied is housed in the herbarium of the National Institute of Polar Research, Tokyo (NIPR).

## 3. Results and Discussion

Living materials of the submerged *Dicranella*-like moss gathered by the senior author were cultured and the chromosomes were observed on new shoots developed from the materials. The somatic chromosome number was observed to be  $n=23$ . The karyological features of this specimen are as follows (Fig. 1): Chromosomes at metaphase varied in length ranging from *ca.* 3 to  $0.5\ \mu\text{m}$ . The smallest chromosome in the complement (Fig. 1 A, B shown in arrows) was stained more lightly than the others at metaphase, which seemed to be the so-called "negative heteropycnosis". According to ONO (1970a, b), this chromosome may be categorized into "m" type hetero-chromosome similar to that in RAMSAY'S report (1974). Therefore, the somatic chromosome number is expressed as  $n=22+m$ . It seems that the chromosome complement consists of two sets of some haploid complement, for example, two satellite chromosomes show similar shape and size to each other. The evidence suggests this specimen is a diploid level species.

There are some studies on karyology of *Dicranella* species. NEWTON (1972) reported  $n=26$  for South Georgian *D. cf. hookeri*, which was the first species known as a diploid level of this genus. All specimens of *D. palustris* studied from temperate regions are known to have chromosome number of  $n=15$  (INOUE, 1974; SMITH and NEWTON, 1968; DANILKIV, 1978). Besides these numbers, 11–16 are known in other species of *Dicranella*. Accordingly, the chromosome number,  $n=23$ , of the Syowa specimen is different from those known in *Dicranella*.

Two species of *Dicranella*, *D. hookeri* and *D. cardotii* are distributed in the mar-

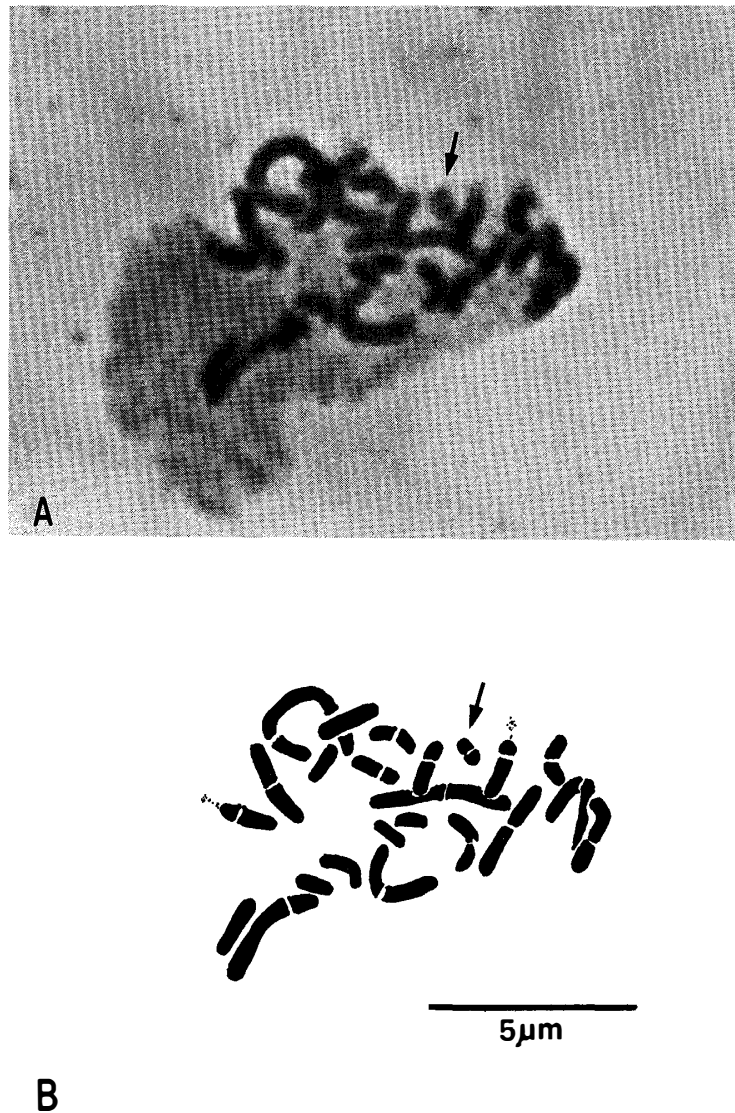


Fig. 1. A: Microphotograph of somatic metaphase chromosomes of *Leptobryum pyriforme* (HEDW.) WILS. showing the number of  $n=22+m$ . Arrow indicates *m* heterochromosome. B: Somatic metaphase chromosomes of *L. pyriforme* (HEDW.) WILS. Arrow indicates *m* heterochromosome.

itime Antarctic. These species are not coincident with the specimen from Syowa Station primarily by the bistratose lamina composed of mamillated cells. As KANDA and IWATSUKI (1989) pointed out, *D. palustris* may be close to the Syowa specimen by such characters as squarrose sheathing bases of leaf, smooth lamina cells of one layer and weakly crenated upper leaf margins. However, the Syowa specimens have more acute leaves with wider costa, forming setaceous leaves at upper stems.

Recently IMURA *et al.* (1992) pointed out that the sexuality of this submerged moss is synoicous by an observation of cultured plants and suggested it to be *Leptobryum pyriforme* (HEDW.) WILS. Morphological features of the submerged moss from Syowa Station are suggestive of the aquatic form of *L. pyriforme* except for smooth rhizoids. *Leptobryum pyriforme* is included in Bryaceae, but is often misidentified as members

of Dicranaceae or Ditrichaceae in case of sterile plants, confused particularly with *Dicranella* and *Ditrichum* (WATSON, 1968; SCOTT and STONE, 1976; CRUM and ANDERSON, 1981). The narrow, almost setaceous leaves, which are reminiscent of *Dicranella*, seem anomalous in the Bryaceae. However, the sporophytic features such as double peristome teeth and well-developed endostome are different from Dicranaceae. Gametophytes of *L. pyriforme* adapted to submerged condition are considered to betray remarkably anomalous forms. Particularly papillae of rhizoids seen in some temperate terrestrial species, which are an important taxonomical character, often become much smaller in water, and disappeared even for a short culture period of two months (HIGUCHI and IMURA, 1987). The smooth rhizoids of the Syowa specimen are assumed to have been caused by a life on lake beds for very long years, so that the specimen may be enough considered to be *L. pyriforme*.

Chromosome numbers of the Antarctic bryaceous plants have been known to be  $n=10$  in *Bryum argenteum*,  $n=20$  in *B. amblyodon*, and  $n=20$  and  $30$  in *B. pseudotriquetrum* (TATUNO, 1963; INOUE, 1976; NEWTON, 1980; KANDA and OKADA, 1990). KANDA and OKADA (1990) discovered a triploid level ( $n=30$ ) of  $x=10$  in one specimen of *B. pseudotriquetrum* from the Syowa Station area, which was the first record of such number for the species. However, the number of  $n=23$  ( $22+m$ ) in the submerged moss is contrary to those of the *Bryum* species.

According to FRITSCH (1991),  $n=20$  (ANDERSON and CRUM, 1958; KHANNA, 1967; SMITH and NEWTON, 1968; BRYAN, 1973),  $21$  (DAVIDSE and POHL, 1974; FETISOVA and VYSOTZKAYA, 1970),  $22$  (LAZARENKO *et al.*, 1970),  $22$  ( $=20+2m$ ) (RAMSAY, 1974),  $24$  ( $=20+4m$ ) (AL-AISH and ANDERSON, 1960) and  $33$  (MAMATKULOV, 1979, 1989) were observed in *L. pyriforme*. Most of the chromosome numbers of *L. pyriforme* vary ranging from  $20$  to  $24$  and the number of  $n=23$  has never been reported in this species.

### References

- AL-AISH, M. and ANDERSON, L. E. (1960): Chromosome numbers of some Arizona mosses. *Bryologist*, **63**, 17–25.
- ANDERSON, L. E. and CRUM, H. A. (1958): Cytotaxonomic studies on mosses of the Canadian Rocky Mountains. *Bull. Natl. Mus. Can.*, **160**, 1–89.
- BRYAN, V. S. (1973): Chromosome studies on mosses from Austria, Czechoslovakia and other parts of Central Europe. *Oesterr. Bot. Z.*, **121**, 187–226.
- CRUM, H. A. and ANDERSON, L. E. (1981): Mosses of Eastern North America. 1. New York, Columbia Univ. Press, 663 p.
- DANILKIV, I. S. (1978): Chromosome numbers of the leafy mosses from the Baltic republic of the USSR. *Ukr. Bot. Zh.*, **35**, 270–272.
- DAVIDSE, G. and POHL, R. W. (1974): Chromosome numbers, meiotic behavior, and notes on tropical American grasses. *Can. J. Bot.*, **52**, 317–328.
- FETISOVA, L. N. and VYSOTZKAYA, E. I. (1970): Chromosome numbers in the mosses from Estonia. *Bot. Zh.*, **55**, 1150–1152.
- FRITSCH, R. (1991): Index to Bryophyte Chromosome Counts. Berlin, J. Cramer, 352 p (Bryophytorum Bibliotheca, Band 40).
- HIGUCHI, M. and IMURA, S. (1987): The effect of submersion on moss rhizoid characters. *Hikobia*, **10**, 59–63.
- IMURA, S. and KANDA, H. (1986): The gemmae of the mosses collected from the Syowa Station area, Antarctica. *Mem. Natl. Inst. Polar Res., Spec. Issue*, **44**, 241–246.

- IMURA, S., HIGUCHI, M., KANDA, H. and IWATSUKI, Z. (1992): Culture of rhizoidal tubers on an aquatic moss in the lakes near the Syowa Station area, Antarctica. *Proc. NIPR Symp. Polar Biol.*, **5**, 114–117.
- INOUE, S. (1974): Chromosomes of a moss ball from the Inawashiro-Lake. *Misc. Bryol. Lich.*, **6**, 141.
- INOUE, S. (1976): Chromosome numbers on five species of Antarctic mosses. *Kumamoto J. Sci. Biol.*, **13**, 1–5.
- KANDA, H. and IWATSUKI, Z. (1989): Two aquatic mosses in the lakes near Syowa Station, Continental Antarctica. *Hikobia*, **10**, 293–297.
- KANDA, H. and MOCHIDA, Y. (1992): Aquatic mosses found in lakes of the Skarvsnes region, the Syowa Station area, Antarctica. *Proc. NIPR Symp. Polar Biol.*, **5**, 177–179.
- KANDA, H. and OHTANI, S. (1991): Morphology of the aquatic mosses collected in lake Yukidori, Langhovde, Antarctica. *Proc. NIPR Symp. Polar Biol.*, **4**, 114–122.
- KANDA, H. and OKADA, H. (1990): Polyploidy in *Bryum* collected from the Syowa Station area, Antarctica. *Nankyoku Shiryô (Antarct. Rec.)*, **34**, 1–7.
- KHANNA, K. R. (1967): A cytological investigation of the mosses of the Rocky Mountains. *Univ. Colo. Stud., Ser. Biol.*, **26**, 1–39.
- LAZARENKO, A. S., VYSOTSKAYA, E. I., LESNYAK, E. N. and MAMATKULOV, U. K. (1970): An investigation of chromosome numbers in the mosses of Tadzhikistan. *Byull., Mosk. Oba. Isprt. Prir., Otd. Biol.*, **75**, 146–155.
- MAMATKULOV, U. K. (1979): Rol' poliploidii v genezise flory listvennykh mkhov Pamiro-Alaja. *Probl. Bot.*, **14**, 95–103.
- MAMATKULOV, U. K. (1989): *Analiz brioflory Pamiro-Alaja*. Dushanbe, Donish, 320 p.
- NAKANISHI, S. (1977): Ecological studies of the moss and lichen communities in the ice-free areas near Syowa Station, Antarctica. *Nankyoku Shiryô (Antarct. Rec.)*, **59**, 68–96.
- NEWTON, M. E. (1972): Chromosome studies in some South Georgian bryophytes. *Br. Antarct. Surv. Bull.*, **30**, 41–49.
- NEWTON, M. E. (1980): Chromosome studies in some Antarctic and sub-Antarctic bryophytes. *Br. Antarct. Sur. Bull.*, **50**, 77–96.
- ONO, K. (1970a): Karyological studies on Mniaceae and Polytrichaceae, with special reference to the structural sex-chromosomes. I. *J. Sci. Hiroshima Univ. Ser. B, Div. 2 (Bot.)* **13**, 91–105.
- ONO, K. (1970b): ditto II. *ibid.* 107–166.
- RAMSAY, H. P. (1974): Cytological studies of Australian mosses. *Austral. J. Bot.*, **22**, 293–348.
- SCOTT, G. A. M. and STONE, I. G. (1976): *The Mosses of Southern Australia*. London, Academic Press, 495 p.
- SMITH, A. J. E. and NEWTON, M. E. (1968): Chromosome studies on some British and Irish mosses. III. *Trans. Br. Bryol. Soc.*, **5**, 463–522.
- TATUNO, S. (1963): Zytologische Untersuchungen über die Laubmoose von Antarktis. *Hikobia*, **3**, 269–274.
- WATSON, E. V. (1968): *British Mosses and Liverworts*. 2nd ed. Cambridge, Cambridge Univ. Press, 495 p.

(Received April 28, 1992; Revised manuscript received August 17, 1992)