J. Jpn. Bot. 85: 118–121 (2010)

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Cytological Studies of the Critically Endangered Plants in Japan (1) *Polemonium kiushianum* (*Polemoniaceae*)

日本の絶滅危惧植物における細胞学的研究（1）ハナシノブ （稲葉一文[a]，的場英行[b]，長野克也[c]，内山 寛[b]）

Summary: *Polemonium kiushianum* Kitam. (*Polemoniaceae*) is a critically endangered species and only a few populations are locally distributed in semi-natural grasslands in the Mt. Aso-san area, Kyushu, Japan. Chromosome number and symmetric karyotype of *P. kiushianum* (2n = 18 = 16m + 2sm) is reported here for the first time. The chromosome measurements and the karyotype formulae showed little difference between *P. kiushianum* and its allied vulnerable species *P. caeruleum* subsp. *yezoense* var. *yezoense*.

Polemonium kiushianum Kitam. (*Polemoniaceae*) is one of the first plants certified by the "Law for the Conservation of Endangered Species of Wild Fauna and Flora" in Japan. This species is also included on the Red List (Environment Agency of Japan 2000) as a critically endangered species. The number of existing individuals is estimated to be about 400. Only eight populations had been locally distributed in semi-natural grasslands in the Mt. Aso-san area (altitude 600–800 m), Kyushu, Japan, but two of them are now recognized as extinct (Environment Agency of Japan 2000, Minamitani 2003).

Polemonium kiushianum is morphologically distinguished from any other taxa in the genus by the following characteristics: raceme-like panicle inflorescences, small corollae (10–15 mm long vs. 18–25 mm long) and small seeds (2 mm long vs. 3–4 mm long) (Kitamura 1941). However, difficulties of classification of *Polemonium* plants has been noted (Hara 1956, Ito 1983) and this species has been treated as a rank of subspecies under *P. caeruleum* (Hara 1956, Yamazaki 1993). Only chromosome number (2n = 18) has been reported in some taxa of Japanese *Polemonium* (*P. caeruleum* subsp. *laxiflorum* f. *paludosum* (Nishikawa 1990, Nishikawa and Ito 1992); *P. caeruleum* subsp. *yezoense* var. *nipponicum* (Nishikawa 1997); *P. caeruleum* subsp. *yezoense* var. *yezoense* (Nishikawa 1990); *P. yesoense* var. *hidakanum* (Nishikawa 1978), while no cytological data is available for *P. kiushianum*.

The aim of this examination is to enhance cytological information of endangered species *Polemonium kiushianum*. In addition, we report the karyotype analysis between *P. kiushianum* and its allied vulnerable species *P. caeruleum* subsp. *yezoense* var. *yezoense*.

We studied chromosomal characteristics from the somatic cells of ten individuals of *Polemonium kiushianum* collected from Takamori, Kumamoto Prefecture and seven individuals of *P. caeruleum* subsp. *yezoense* var. *yezoense* obtained from a supplier (originally from Sapporo). The voucher specimens were deposited in the herbarium of Makino Botanical Garden (MBK).

Root tips were pretreated with 2 mM 8-hydroxyquinoline at 0°C for 12 hours before being fixed in 45% acetic acid at room temperature for two hours. Fixed root tips were macerated in 1N HCl for 2min at 60°C. After washing with distilled water for 1 h, they were stained with paraldehyde fuchsin. Slide preparations were made by the conventional squash method. Chromosomal measurements

Table 1. Chromosome measurements of *Polemonium kiushianum* and *P. caeruleum* subsp. *yezoense* var. *yezoense*

Number	*P. kiushianum*				*P. caeruleum* subsp. *yezoense* var. *yezoense*			
	Length (μm)	Total (μm)	Arm ratio	Type	Length (μm)	Total (μm)	Arm ratio	Type
1	2.6 + 2.9	5.5	1.1	m	2.8 + 2.8	5.6	1.0	m
2	2.6 + 2.8	5.4	1.1	m	2.7 + 2.9	5.6	1.1	m
3	2.4 + 2.9	5.3	1.2	m	2.4 + 3.0	5.4	1.3	m
4	2.4 + 2.9	5.3	1.2	m	2.4 + 3.0	5.4	1.3	m
5	2.2 + 3.0	5.2	1.4	m	2.4 + 2.9	5.3	1.2	m
6	2.2 + 2.9	5.1	1.3	m	2.3 + 3.0	5.3	1.3	m
7	2.3 + 2.7	5.0	1.2	m	2.3 + 2.8	5.1	1.2	m
8	2.3 + 2.7	5.0	1.2	m	2.2 + 2.7	4.9	1.2	m
9	2.2 + 2.7	4.9	1.2	m	2.2 + 2.7	4.9	1.2	m
10	2.3 + 2.6	4.9	1.1	m	2.2 + 2.7	4.9	1.2	m
11	2.1 + 2.8	4.9	1.3	m	2.1 + 2.4	4.4	1.1	m
12	2.0 + 2.8	4.8	1.4	m	2.0 + 2.4	4.4	1.2	m
14	2.0 + 2.6	4.6	1.3	m	1.9 + 2.4	4.3	1.3	m
15	2.0 + 2.6	4.6	1.3	m	1.8 + 2.4	4.2	1.3	m
13	1.9 + 2.7	4.6	1.4	m	1.9 + 2.3	4.1	1.2	m
16	1.8 + 2.6	4.4	1.4	m	1.8 + 2.3	4.0	1.3	m
17	1.4 + 2.4	3.8	1.7	sm	1.4 + 2.4	3.8	1.7	sm
18	1.4 + 2.4	3.7	1.7	sm	1.3 + 2.2	3.5	1.7	sm

were made from microscope images of at least ten well spread metaphase plates taken by Sensys cooled CCD camera (Photometrics). Chromosomes at mitotic metaphase were classified by arm ratio following Levan et al. (1964).

The chromosome numbers were counted as 2n = 18 in both species investigated. Chromosomal measurements and karyotypes for each species are shown in Table 1 and Fig. 1, respectively. The somatic chromosome number of P*olemonium kiushianum* was 2n = 18 (Fig. 1A). The chromosomes of this species indicated a gradual decrease in length from the largest (5.8 μm) to the shortest (4.0 μm) chromosomes and consisted of 16 median- and 2 submedian-centromeric chromosomes (Table 1, Fig. 1C). The submedian chromosomes were smaller than the median chromosomes. No satellite was observed. The chromosome number and karyotype are the first report for this species.

The chromosome number of *Polemonium caeruleum* subsp. *yezoense* var. *yezoense* was 2n = 18 (Fig. 1B). The chromosomes of this species showed a gradual decrease in length from the largest (6.3 μm) to the shortest (3.6 μm) chromosomes and consisted of 16 median- and 2 submedian-centromeric chromosomes (Table 1, Fig. 1D). The submedian chromosomes were smaller than the median chromosomes. No satellite was observed. This chromosome number agreed with the previous report (Nishikawa 1990), but the karyotype is reported here for the first time.

The chromosomes of *Polemonium* examined show a symmetric karyotype and are nearly uniform in size and shape, resulting in impaired discrimination of homologous chromosomes. Moreover, the chromosome measurements and the karyotype formulae (in the order of chromosome length: 2n = 18 = 16m + 2sm) showed little difference between *P. kiushianum* and *P. caeruleum* subsp. *yezoense* var. *yezoense*, thus it is difficult to distinguish between them by the karyotype analysis.

We thank Mr. Norimitsu Uno of Hana-

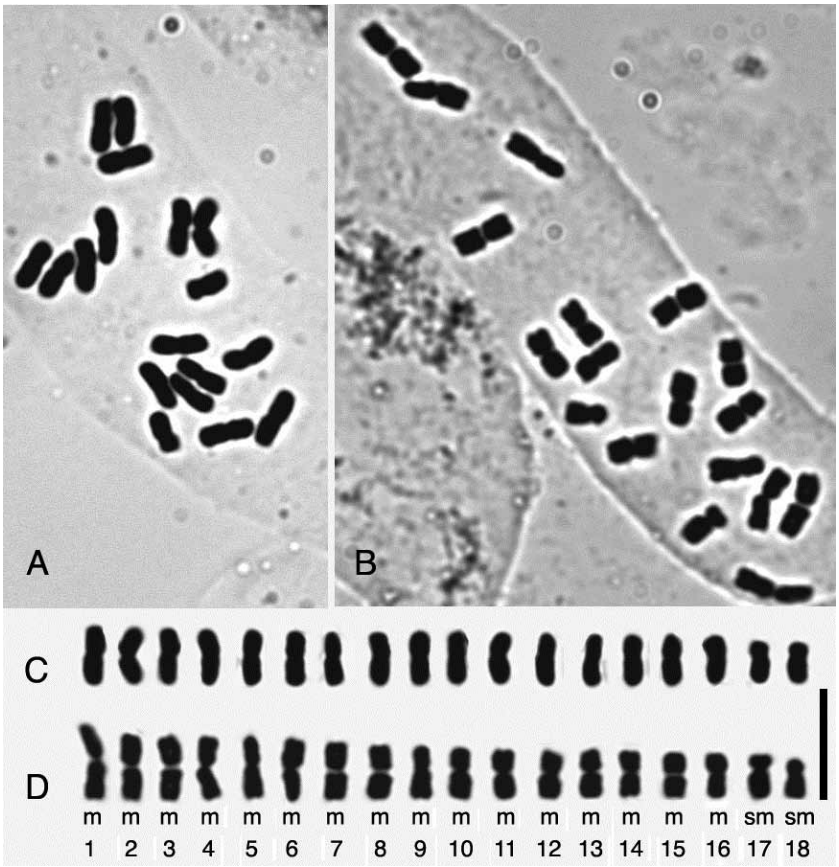

Fig. 1. Chromosomes at mitotic metaphase (A, B) and karyotypes (C, D) of *Polemonium kiushianum* and *P. caeruleum* subsp. *yezoense* var. *yezoense*. A, C. *P. kiushianum* (2n = 18 = 16m + 2sm). B, D. *P. caeruleum* subsp. *yezoense* var. *yezoense* (2n = 18 = 16m + 2sm). The karyotype formula is in the order of chromosome length. Scale bar represents 10 μm for A to D.

sakimori for his helpful support of sampling. Plant materials were collected with the permission of Ministry of the Environment. This study was supported by Grants-in-Aid for Scientific Research (20917005, 19915004, 18915009 and 17915013) from the Japan Society for the Promotion of Science (K. I.).

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ハナシノブ *Polemonium kiushianum* Kitam. は，日本で初めて“絶滅のおそれのある野生動植物の種の保存に関する法律”が適用された絶滅危惧植物の一種で，阿蘇山周辺の半自然草原に数個体群のみが分布している．ハナシノブの染色体数及び核型（2n = 18 =16m + 2sm）は今回初めて報告されるものである．染色体測定及び核型の観察結果は，ハナシノブとそれに近縁な危急種のエゾハナシノブ *P. caeruleum* subsp. *yezoense* var. *yezoense* にほとんど違いがないことを示した．

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J. Jpn. Bot. 85: 121–125 (2010)

Norihito MIURA[a] and Yoshikane IWATSUBO[b]: **Karyotype of *Coleus formosanus* (*Labiatae*)**

ケサヤバナ（シソ科）の核型 （三浦憲人[a]，岩坪美兼[b]）

Summary: We examined the karyotype of *Coleus formosanus* Hayata (*Labiatae*) collected from Yonaguni Island, Okinawa Prefecture. This plant had 2n = 50 chromosomes, confirming the count reported previously from Taiwan (Hsieh and Huang 1998). The metaphase chromosomes ranged from 1.1 μm to 2.5 μm in length and 1.0 to 2.3 in arm ratio. The longest four pairs were all submetacentric and the other 21 pairs were all metacentric. The karyotype is formulated as 2n = 50 = 42m + 4sm + 2^{sc}sm + 2^{t}sm, and shows that this species has a new basic chromosome number of x = 25.

Coleus formosanus Hayata (*Labiatae*) is a perennial herb distributed between Japan, Taiwan and the Philippines. In Japan this species occurs only in Yonaguni Island, Okinawa Prefecture (Murata and Yamazaki 1993). Chromosome number of this species is reported to be 2n = 50 in Taiwanese *C. formosanus* (Hsieh and Huang 1998). As listed in Table 1, the genus *Coleus* has various chromosome numbers of n = 12, 14, 15, 16, 17, 24, 25, 34, and 36, and 2n = 24, 28, 30, 32, 34, 48, 49, 50, 51, 52, 56, 60 and 64. On the basis of these counts, basic chromosome numbers of this genus had been proposed as x = 6, 7, 8, 9, 12, 14, 15, 16, 17 and 18 (Darlington and Wylie 1955, Bir and Saggoo 1982, Gill 1984, Singh 1995). The somatic chromosome count of 2n = 50 in *C. formosanus* reported by Hsieh and Huang (1998), however, does not