



Somatic chromosome numbers of four *Eleocharis* taxa from Korean populations (Cyperaceae)

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ABSTRACT: *Eleocharis* R. Br. (Cyperaceae) is characterized by unbranched culms, one terminal spikelet, bisexual flowers, and bristly perianths, occurring mainly in circumboreal marshes and wetlands. There are about 250 species worldwide, and 15 taxa in Korea have been recognized. Chromosomes in Cyperaceae are known to be holocentric, missing constricted centromeres during cell division. For the first time, we report the chromosome counts of *Eleocharis* from Korean populations of *E. attenuata* f. *laeviseta* (Nakai) H. Hara ($2n = 20$); *E. parvula* (Roem. & Schult.) Link ex Bluff, Nees & Schauer ($2n = 10$); *E. ussuriensis* Zinslerl. ($2n = 16$); and *E. valleculosa* var. *setosa* Ohwi ($2n = 16$). The populations exhibit variations in their chromosome sizes from ca. 1.7 μm to ca. 6 μm , categorized as gradient and bimodal karyotypes. The karyotypes observed are congruent with previous phylogenetic groups proposed. To clarify the traditional and phylogenetic classification of Korean *Eleocharis*, further taxonomic and chromosomal investigations with additional taxon sampling efforts are required.

Keywords: chromosome number, Cyperaceae, *Eleocharis*, holocentric chromosome

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INTRODUCTION

Eleocharis R. Br. is characterized by unbranched culms, one terminal spikelet, bisexual flowers, and bristly perianths (Smith et al., 2002; Dai and Strong, 2010; Park et al., 2016). It occurs mainly in circumboreal marshes and wetlands, and includes about 250 species (González-Elizondo and Peterson, 1997; Smith et al., 2002; Dai and Strong, 2010). González-Elizondo and Peterson (1997) recognized four subgenera, seven sections, eight series, and seven subseries. In Korean *Eleocharis*, Oh (2007) and Park et al. (2016) recognized 13 or 15 taxa, respectively. Oh and Lee (1997) and Oh (2000) classified Korean taxa into two sections (*Limnochloa* (P. Beauv. & T. Lestib.) Benth. & Hook. f. and *Eleocharis* R. Br.) based on spikelet and achene sizes.

Eleocharis possesses holocentric chromosomes (diffuse chromosomes), which miss constricted centromeres during cell divisions (Håkansson, 1954, 1958; Hoshino, 1987; Yano et al., 2004). Chromosome numbers in the genus range broadly from $2n = 10$ to $2n = 196$ ($x = 5$), and mixoploidy is common

(Hoshino, 1987; Hoshino et al., 1993; Yano et al., 2004). The sizes of chromosomes also vary in the genus from less than 0.7 μm to about 6 μm (Hoshino, 1987; Hoshino et al., 1993; Yano et al., 2004). *Eleocharis* taxa with small chromosome numbers generally have large chromosomes such as *E. valleculosa* f. *setosa* Ohwi ($2n = 16$, ca. 3–6 μm in length) whereas taxa with large chromosome numbers exhibit small chromosomes as in *E. dulcis* (Burm. f.) Trin. ex Hensch. ($2n =$ ca. 196, less than 1 μm in length) (Hoshino et al., 1993; Yano et al., 2004). In addition, satellites were observed in some taxa, *E. mamillata* var. *cycrocarpa* Kitag. and *E. acicularis* (L.) Roem. & Schult. f. *longiseta* (Svenson) T. Koyama (Hoshino et al., 1993; Yano et al., 2004).

Out of 15 Korean taxa, none of them has chromosome data reported. However, a few *Eleocharis* taxa from Chinese and Japanese populations have been reported (Hoshino, 1987; Hoshino et al., 1993; Yano et al., 2004; Hoshino et al., 2011). *E. mamillata* var. *cycrocarpa* has chromosome numbers of $2n = 16$ commonly in Chinese and Japanese populations, but two satellites are observed only in Chinese populations (Hoshino

et al., 1993). This suggests chromosomal diversification within a taxon. Intraspecific diversity in chromosome number and configuration are related with diffuse centrometric chromosomes (Hoshino et al., 1993; Yano et al., 2004). *Eleocharis* chromosome data analyzed in a phylogenetic framework suggest that fragmentation (fission), fusion and polyploid events contributed to the species diversity in the genus (Yano et al., 2004).

In the previous investigations, several types of chromosomes have been recognized in *Eleocharis*. Hoshino (1987) recognized three types of chromosomes in *Eleocharis*: (1) round prochromosome type (bimodal variation) (*E. mamillata* var. *cyclacarpa*), (2) complex chromocenter type (gradational variation) (*E. wichurae* Boeckeler, *E. tetraquetra* Nees, *E. acicularis* (L.) Roem. & Schult., and *E. congesta* var. *thermalis* (Hultén) T. Koyama), and (3) sparsely diffuse type (very small, under 0.7 μm in length). Variation in chromosome numbers has also been reported in the genus and also in other genera in Cyperaceae. The chromosome variation in size and number is hypothesized in a phylogenetic framework by Yano et al. (2004). There are three types of chromosome groups supported by phylogenetic data: (1) very small chromosomes in section *Limnochloa* (*E. dulcis*, *E. kuroguwai* Ohwi), (2) gradient variation in size in sections *Pauciflorae* (Beauverd) Svenson and *Eleocharis*, and (3) two-sized chromosomes, bimodal karyotypes (large and small) in series *Eleocharis*.

We report somatic chromosome numbers of four Korean native *Eleocharis* taxa: *E. attenuata* f. *laeviseta* (Nakai) H. Hara, *E. parvula* (Roem. & Schult.) Link ex Bluff, Nees & Schauer, *E. ussuriensis* Zinserl. and *E. valleculosa* var. *setosa* Ohwi.

Significance of their chromosome numbers is also discussed.

MATERIALS AND METHODS

Root tips were sampled in natural habitats during the field surveys in various collection sites (Table 1). Initially, root tips were pretreated in 0.002 M 8-hydroxyquinoline for over 8 h and then fixed in a mixture of ethanol and glacial acetic acid (3:1) for 12 h. After fixation, root tips were preserved in 70 % ethanol, then macerated in 1 N mol HCl for 10 min before 1 % acetic-orcein staining. Stained root tips were squared and observed at 1,000 \times magnification (Nikon Eclipse 50i, Nikon, Tokyo, Japan). At least three mitotic cells per individual were analyzed to confirm numbers and types of chromosomes. Drawing and photographs were made for the cells analyzed. Park et al. (2016) was used for identification, and scientific and Korean names followed those at the Korea National Institute of Biological Resources species database. Voucher specimens were saved in the Korea National Institute of Biological Resources herbarium (KB).

RESULTS AND DISCUSSION

In nine individuals of four *Eleocharis* taxa, somatic chromosomes were observed (Table 1). In metaphase chromosomes, constrictions were not observed as expected in holocentric chromosomes (Chung and Chung, 2021). The size varies from ca. 1.7 μm to ca. 6 μm . *E. attenuata* f. *laeviseta* and *E. parvula* seem to have more than two types of

Table 1. *Eleocharis* taxa investigated with voucher specimens and chromosome numbers.

Taxon (locality, voucher specimen)	2n	Previous counts (2n)
<i>E. attenuata</i> f. <i>laeviseta</i> (Nakai) H. Hara 참바늘골 Korea. Jeju-do, Jeju-si, Jocheon-eup, Seonheullam-4-gil, Dongbaengno (Chung & Lee 9464, 17 Jul 2022)	20	None
Korea. Jeju-do, Seogwipo-si, Pyoseon-myeon, Seongeup-ri (Chung & Lee 9485, 19 Jul 2022)	20	
<i>E. parvula</i> (Roem. & Schult.) Link ex Bluff, Nees & Schauer 갯바늘골 Korea. Incheon-si, Ganghwa-gun, Hwado-myeon, Sagi-ri (Chung et al. 220919-1, 19 Sep 2022)	10	10 (Fedorov, 1969; Yano et al., 2004); 8 (Fedorov, 1969)
(Chung et al. 220919-2, 19 Sep 2022)	10	
<i>E. ussuriensis</i> Zinserl. 물꼬챙이골 Korea. Gyeongsangbuk-do, Mungyeong-si, Sanbuk-myeon, Ugok-ri (Chung & Lee 9231, 22 Jun 2022)	16	16 (Shatalova, 1999, 2000); 16 (Hoshino, 1987; Hoshino et al., 1993 reported as <i>E. mamillata</i> var. <i>cyclacarpa</i>); 20 (Hoshino et al., 2011 reported as <i>E. mamillata</i> var. <i>cyclacarpa</i>)
(Chung & Lee 9233, 22 Jun 2022)	16	
<i>E. valleculosa</i> var. <i>setosa</i> Ohwi 가락골 Korea. Jeju-do, Jeju-si, Jocheon-eup, Seonheullam-4-gil, Dongbaengno (Chung & Lee 9466, 17 Jul 2022)	16	16 (Hoshino et al., 1993)
Korea. Jeju-do, Jeju-si, Jocheon-eup, Seonheullam-4-gil, Dongbaengno (Chung & Lee 9474, 17 Jul 2022)	16	
Korea. Jeju-do, Seogwipo-si, Pyoseon-myeon, Seongeup-ri (Chung & Lee 9484, 19 Jul 2022)	16	

chromosomes in size, whereas *E. ussuriensis* and *E. valleculosa* var. *setosa* exhibit bimodal karyotypes.

Eleocharis attenuata f. *laeviseta* H. ($2n = 20$)

Eleocharis attenuata f. *laeviseta* from two Jeju Is. populations exhibits the chromosome numbers of $2n = 20$ (Fig. 1A, B), which is the first report for the taxon. The size of chromosomes varies along a gradient from ca. 1.7 μm to ca. 4.5 μm . The count is congruent with the chromosome number of *E. attenuata* f. *attenuata* (Yano et al., 2004). The taxon occurs in Korea, China, Japan, Russia, Papua New Guinea, and Vietnam, and southern parts of Korean peninsula,

Jeollanam-do province and Jeju Is. (Park et al., 2016). *Eleocharis attenuata* f. *laeviseta* is distinguished from *E. attenuata* f. *attenuata* by depressed style bases and smooth perianth bristles (Hoshino et al., 2011; Park et al., 2016).

Korean name: 참바늘골.

Eleocharis parvula ($2n = 10$)

We observed $2n = 10$ chromosome numbers from two populations of *E. parvula* in Korea (Fig. 1C, D). The size of chromosomes is ca. 3–4 μm . Previously, two chromosome numbers for the species have been reported from Russian and Japanese populations, $2n = 8$ and $2n = 10$ (Fedorov, 1969;

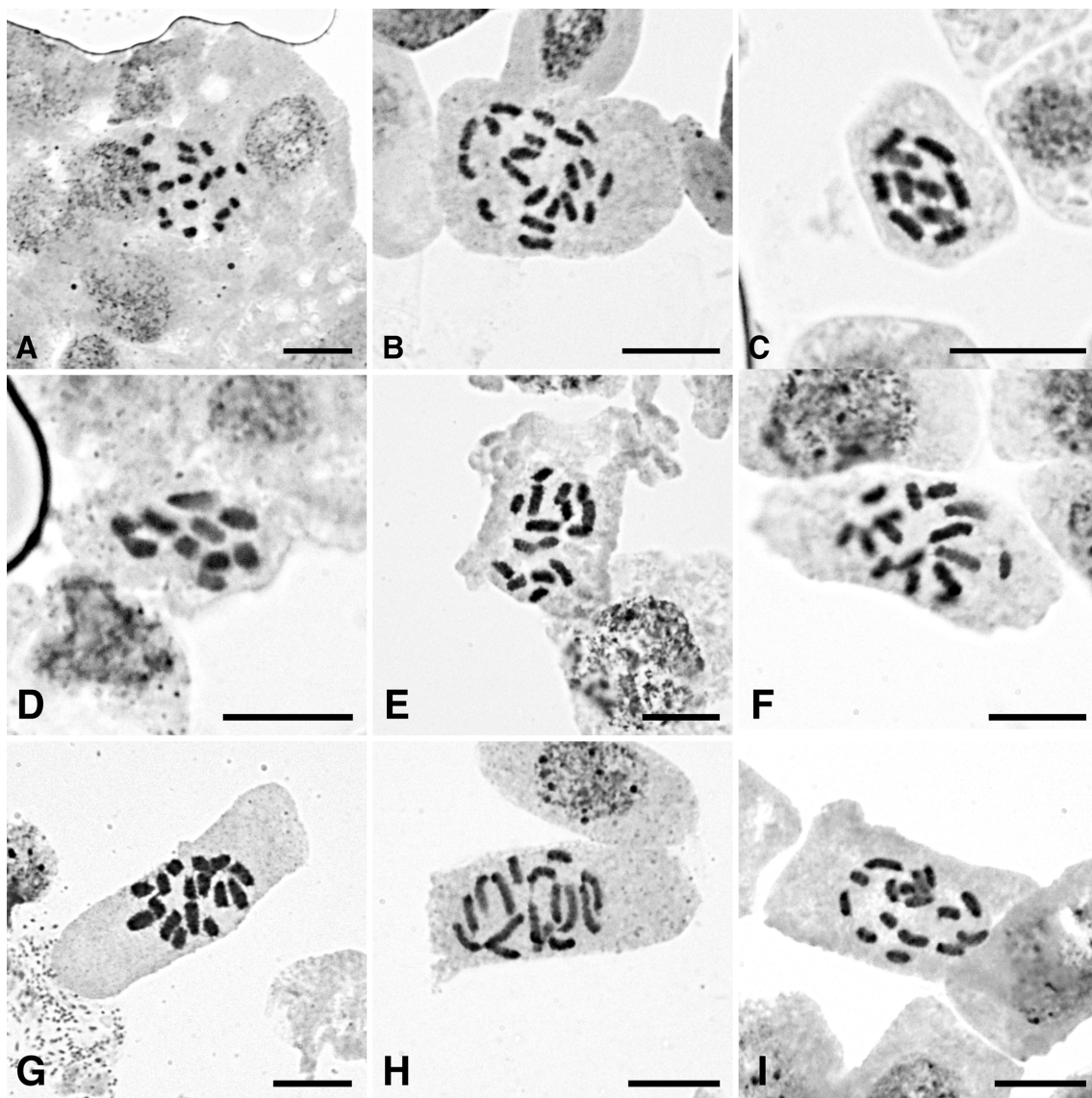


Fig. 1. Photomicrographs of *Eleocharis* somatic chromosomes. **A.** *E. attenuata* f. *laeviseta* ($2n = 20$, Chung and Lee 9464). **B.** *E. attenuata* f. *laeviseta* ($2n = 20$, Chung and Lee 9485). **C.** *E. parvula* ($2n = 10$, Chung et al. 220919-1). **D.** *E. parvula* ($2n = 10$, Chung et al. 220919-2). **E.** *E. ussuriensis* ($2n = 16$, Chung & Lee 9231). **F.** *E. ussuriensis* ($2n = 16$, Chung & Lee 9233). **G.** *E. valleculosa* var. *setosa* ($2n = 16$, Chung & Lee 9466). **H.** *E. valleculosa* var. *setosa* ($2n = 16$, Chung & Lee 9474). **I.** *E. valleculosa* var. *setosa* ($2n = 16$, Chung & Lee 9484). Scale bars = 10 μm .

Yano et al., 2004). The species occurs worldwide in Europe, Siberia, N. Africa, Central, North and South America, and Asia, and habitats are limited to marshes along seacoasts (Dai and Strong, 2010; Hoshino et al., 2011). Recently, Korean populations have been reported from Ganghwa-gun, Incheon Metropolitan City (Ji et al., 2015). The species is characterized by small fusiform tubers, septa-less culms, and confluent style bases with achene apexes, and occurs in salt marshes (Hoshino et al., 2011; Ji et al., 2015).

Korean name: 갯바늘골.

Eleocharis ussuriensis ($2n = 16$)

Somatic chromosomes of $2n = 16$ were observed from the species, confirming previous reports (Fig. 1E, F, Table 1). The size of chromosomes ranges from ca. 2.5 μm to 5 μm in length, grouped in two sizes ca. 2.5–3 μm and ca. 4–5 μm . In the species, Hoshino et al. (1993) observed bimodal karyotypes (small and large) in the species and found satellites in small chromosomes (2.5–3.5 μm in length) from a Chinese population, but they were not found in the Japanese population. Hoshino et al. (2011) also reported the chromosome number of $2n = 20$ for the species. However, we are unable to discuss the data because the original article misses voucher species information and chromosome figures. In the chromosomes from Korean populations, satellites were not observed. The species is characterized by long rhizomes and large achenes (1.5–2 μm long), and occurs in Japan, Korea, Sakhalin, Ussuri, and N. E. China (Hoshino et al., 2011). In Korea, it is found mainly in central areas (Park et al., 2016). The taxon has been treated as *E. mamillata* var. *cyclacarpa* in Japan and Korea (Hoshino et al., 2011; Park et al., 2016). However, we used *E. ussuriensis* based on the principle of publication priority (Dai and Strong, 2010).

Korean name: 물꼬챙이골.

Eleocharis valleculosa var. *setosa* ($2n = 16$)

From three populations in Jeju Is., somatic chromosomes of *E. valleculosa* var. *setosa* were observed, $2n = 16$ (Fig. 1G–I). The size of the chromosomes varies from ca. 3 μm to ca. 6 μm in length, exhibiting bimodal karyotypes, four or five large and the other small ones. The chromosome number agrees with the count made from a Chinese population (Hoshino et al., 1993). *E. valleculosa* var. *valleculosa* also has chromosome of $2n = 16$ counted from a Japanese population (Yano et al., 2004). The species is characterized by sharp ribs (striate) on culms, oblong spikelets, and four long perianth bristles, growing in shallow waters such as moors and margins of ponds in mountains (Hoshino et al., 2011; Park et al., 2016). It occurs in Korea, China, Japan, and Mongolia (Hohisno et

al., 2011). During the research, we examined *Eleocharis* collections in major herbaria such as Korean National Arboretum and Korea National Institute of Biological Resources herbaria to find *E. valleculosa* var. *valleculosa*, which misses perianth bristles. All the herbarium specimens collected and examined were identified as *E. valleculosa* var. *setosa*.

Korean name: 까락골.

In four Korean *Eleocharis* taxa, we observed at least two types of karyotypes, gradient and bimodal. Each karyotype was supported by molecular data although taxon and DNA sampling was limited (Yano et al., 2004). Chromosome numbers of $2n = 10, 16, 20$ in *Eleocharis* suggest polyploidy, aneuploidy, fission and/or fusion events. Because of specialized and reduced morphology and holocentric chromosomes, it is critical to have inclusive taxon and DNA sampling to test traditional classification in a phylogenetic framework. Roalson and Friar (2000) and Yano et al. (2004) attempted to estimate *Eleocharis* phylogenetic relationships, but the taxon sampling was too limited to apply to the whole genus. Further chromosomal investigation of Korean *Eleocharis* research should contribute to elucidating phylogenetic relationships of the genus.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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