Cytotype distribution and ecology of *Allium thunbergii* (= *A. sacculiferum*) with a special reference to South Korean populations

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ABSTRACT: Polyploidization plays an important role in generating the current high diversity of plants. Studies of the distributional patterns of diploid and derivative polyploid races have provided important insights into the evolutionary process and cryptic speciation by polyploidization within and between closely related taxa defined on the basis of their morphology. *Allium thunbergii* and *A. sacculiferum*, occurring throughout eastern Russia, eastern China, Korea, and Japan, are examples of closely related species with unsolved taxonomic relationships. A total of 97 and 65 individuals from 26 and 13 populations of *A. thunbergii* (including var. *thunbergii*, var. *deltoides*, and var. *teretifolium*) and *A. sacculiferum*, respectively, were studied to determine their ploidy. The geographic structure and habitat differentiation of the cytotypes were also analyzed. The main cytotype of *A. thunbergii* was diploid (92.3% in total; the rest were tetraploids). In contrast, the majority of *A. sacculiferum* plants were tetraploids (69.2% of the total; the rest were diploids). No populations of the studied taxa harbored both cytotypes. *Allium thunbergii* was more often found at higher elevations than *A. sacculiferum*, and it tended to occur more frequently on rocky slopes and below forests in mountainous areas. On the other hand, *A. sacculiferum* occurred at forest margins and in lowland pastures. The cytotypes differed with respect to the elevation; diploids were found more frequently at higher elevations than tetraploids. The results of this study and additional biosystematics data indicate that the morphological characteristics of *A. thunbergii* and *A. sacculiferum* may be influenced by polyploidization and by their adaptation to various habitat conditions and that *A. thunbergii* and *A. sacculiferum* do not clearly fulfill the requirements of any species concept. Consequently, we propose that *A. sacculiferum* be considered as an additional synonym of *A. thunbergii*. Additionally, *Allium thunbergii* var. *deltoides* is unified into *A. thunbergii* var. *thunbergii*.

Keywords: *Allium thunbergii*, *Allium sacculiferum*, cytotype, polyploidy, habitat differentiation

Polyploidy has long been recognized as an important mechanism of the plant evolution (Grant, 1981; Soltis et al., 1993; Thompson et al., 1997; Ramsey and Schemske, 1998; Peirson et al., 2012), driving genomic novelty and acting as one of the primary mechanism of sympatric speciation (Otto and Whitton, 2000; Wood et al., 2009). Besides harmful effects of polyploidy on fertility and fitness due to mitotic and meiotic abnormalities, and genomic instability (Comai, 2005), polyploidy has also adaptive potential allowing some new

polyploids to survive and evolutionary diverge (Soltis et al., 2009; te Beest et al., 2012). Genome duplication is often accompanied by a creation of reproductive barriers through multiple mechanisms including a transition from cross-pollination to self-pollination or even from sexual to asexual reproduction, shifts in phenology, and spatial segregation (Comai, 2005; Kolář et al., 2017; van der Peer et al., 2017).

Increased genome size and genetic variation can also potentially affect the morphology and physiology of newly

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formed polyploids, and these changes might lead to niche differentiation (Ramsey, 2011; te Beest et al., 2012; Madlung, 2013; Sonnleitner et al., 2016). Adaptive potential of polyploids is, however, expected to depend on the route of polyploid origin. Polyploids are traditionally classified into the two major types, i.e., autopolyploids and allopolyploids (Kihara and Ono, 1926). It has been supposed that allopolyploidy that include two steps, hybridization and genome duplication, provide the polyploid an initial selective advantage because hybridizing species are already diverged (Comai, 2005). However, polyploidization both in allo- and autopolyploids can also lead to changes in gene expression and consequently to changes in ecology and it seems presently that these changes are rather species-dependent (van der Peer et al., 2017).

Owing to increased genetic variation in polyploids, it has been repeatedly proposed and experimentally proven that polyploid might tolerate newly created, disturbed or harsh environments (Ramsey, 2011; Manzaneda et al., 2012; Hao et al., 2013; Madlung, 2013). Indeed, it has been evidenced many times that polyploids are ecologically distinct from their diploid relatives and often occupy at least partially separate geographic ranges (e.g., Müntzing, 1936; Grant, 1981; Xie-Kui et al., 2008; Šafářová et al., 2011). However, several recent papers on large data sets questioned evidences for the supposed broader ecological tolerances or increased tolerance of polyploids to harsher environments because in most studied examples high niche overlap or absence of niche shifts between diploids and polyploids were found (Glennon et al., 2014; Marchant et al., 2016). It is clear that whether polyploidy promotes niche differentiation require further research (Parisod and Broennimann, 2016).

Allium L. is one of the largest genera of monocots (Friesen et al., 2006; Li et al., 2010). Currently, the number of species within the genus is estimated at 920 (Seregin et al., 2015). Mixed-ploidy species—taxa containing at least two cytotypes—represent 16% of all plant species based on chromosomal surveys (Rice et al., 2015) while their proportion comprise ca. 19% in the genus *Allium* (Hanelt et al., 1992). Understudied cytotype variation can lead to the underestimation of species richness (Soltis et al., 2010) and incorrect estimation of niche breadth and geographic distribution (Wefferling et al., 2017). A better understanding of polyploid complexes or closely related taxa differing in ploidy level thus require basic cytogeographic data that help together with morphological data to resolve taxonomic issues and even enhance genetic approaches that may be confounded by cryptic and frequently also reticulate speciation (Soltis et al., 2007; Suda et al., 2007; Grusz et al., 2009).

One example of closely related taxa differing in ploidy level with hitherto unsolved taxonomy and limited knowledge of cytotype composition and distribution pattern are *Allium thunbergii* G. Don and *A. sacculiferum* Maxim., belonging to section *Sacculiferum* P. P. Gritz., of subgenus *Cepa* (Mill.) Radic (Friesen et al., 2006; Li et al., 2010) that occur throughout Eastern Russia, Eastern China, Korea, and Japan (Choi and Oh, 2011). Although both species do not have any information about ploidy levels by the original authors, *A. thunbergii*, originally described from Japan, was reported its chromosome numbers to $2n = 16$ ($2x$), 32 ($4x$), or 48 ($6x$) in Japan (Kurita, 1952; Noda and Watanabe, 1967; Hoshiya, 1982; Hotta, 1998), while *A. sacculiferum* was described from Russia (Far East) and known as a tetraploid species of $2n = 32$ in Russia (Probatova et al., 2013). It is still unclear if these closely related taxa could be considered as two separate species, especially there has been much confusion between *A. thunbergii* var. *thunbergii* and *A. sacculiferum* (Choi and Oh, 2011). These species exhibit a wide range of morphological variation even among individuals within populations (Yu et al., 1981a; Choi and Oh, 2011). Choi and Oh (2011) distinguished the latter from the former by several morphological characters (clearly elongated leaf sheath, which is usually exposed above ground, and longer scape), and different chromosome numbers: *A. thunbergii* is a diploid with $2n = 16$, whereas *A. sacculiferum* is a tetraploid with $2n = 32$. However, Choi and Oh (2011) also noticed a diploid population that, based on morphological observations, belongs within the range of tetraploid *A. sacculiferum*.

The goals of this study are (1) to broaden the current knowledge on cytogeography and ecology (in addition to Choi and Oh, 2011) of *A. thunbergii* and *A. sacculiferum*, especially focused on the South Korean populations; (2) to examine whether diploid and tetraploid populations show segregation in relation to the elevational gradient or habitat types; and (3) to address taxonomic confusion between *A. thunbergii* and *A. sacculiferum*.

Materials and Methods

The collected samples were tentatively morphologically identified based of Choi and Oh (2011), and details of sampled populations are listed in Table 1. The coordinates and elevations were recorded with a Garmin GPS 60CSx instrument (Taipei, Taiwan). To determine chromosome numbers, shoot tips were pretreated in 0.002 M 8-hydroxyquinoline for 4–6 h in darkness at 4°C and then fixed in Carnoy's fluid (3 parts absolute ethanol:1 part glacial acetic

Fig. 1. Metaphase chromosomes of *Allium thunbergii* (A, var. *thunbergii*; B, var. *deltoides*; C, var. *teretifolium*) and *A. sacculiferum* (D, E). Arrow, satellite chromosome; b1, B-chromosome. Scale bar = 10 μ m (Revised from Fig. 4 of Ko et al. 2009).

acid, v/v) for 1 h at room temperature (23°C). The shoot tips were macerated in 1M hydrochloric acid at 60°C for 10–15 s. After washing 3–5 times to eliminate residual hydrochloric acid and staining with 1% aceto-orcein for 8 h, the material was squashed for observation in 45% acetic acid. More than 10 micrographs were taken for each accession by an optical microscope (Olympus AX-70, Tokyo, Japan) (Fig. 1). Semi-permanent microscope slides and photographs of representative cells have been retained in the Plant Systematics Laboratory of the Changwon National University, South Korea. We used Mann-Whitney U-test to test differences in elevation between di- and tetraploids.

Results

A total of 97 and 65 individuals from 26 and 13 populations of tentatively morphologically identified *A. thunbergii* (including var. *thunbergii*, var. *deltoides*, and var. *teretifolium*) and *A. sacculiferum* were counted for chromosome numbers,

respectively (Fig. 1, Table 1). Only single-ploidy populations were found in both species (mean = 4.7 and 5 samples per population in *A. thunbergii* and *A. sacculiferum*, respectively) (Fig. 2, Table 1). The most frequent cytotype of *A. thunbergii* was diploid ($2n = 16$) found in 24 out of 26 populations (92.3%) while rare tetraploid cytotype ($2n = 32$) was found in remaining 2 populations. Most *A. sacculiferum* populations (69.2%) were tetraploid including two populations from eastern Russia and China and remaining populations were diploid (Table 1).

Most diploids of both species were found at higher elevations above ca 900 up to 1,700 m, whereas tetraploids of both species tend to occur below 800 m. However, some diploid populations were also found at relatively low elevations (Fig. 3). Median elevation of diploids (876 m) was significantly higher than that of tetraploids (268 m) (Mann-Whitney U-test, $Z = -3.17$, $p = 0.002$) (Fig. 3).

Habitat preferences of the cytotypes of two species were analyzed (Fig. 4). *Allium sacculiferum* occurs commonly in

Table 1. The population locations and chromosome numbers (CN) with numbers of examined individuals (NI) of *Allium thunbergii* and *A. sacculiferum*.

Pop	Locality	Geographical position	Elev. (m)	Habitat	CN (NI)	Voucher
<i>A. thunbergii</i> var. <i>thunbergii</i>						
1	Hwaaksan Mt., Cheongdo-gun, Gyeongbuk, KOREA	35°35'25.1"N, 128°41'35.8"E	906	Forest in mountain	2n = 16 (5)	Choi & Jang TH-01
2	Daedeoksan Mt., Gimcheon-si, Gyeongbuk, KOREA	35°56'07.9"N, 127°53'47.6"E	695	Forest in mountain	2n = 16 (4)	Choi & Jang TH -02
3	Yongmunsan Mt., Yangpyeong-gun, Gyeonggi, KOREA	37°33'43.4"N, 127°33'06.2"E	1,023	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -03
4	Seodaesan Mt., Geumsan-gun, Chungnam, KOREA	36°13'29.8"N, 127°32'34.2"E	845	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -04
5	Songnisan Mt., Boeun-gun, Chungbuk, KOREA	36°32'52.6"N, 127°51'32.0"E	563	Rocky slope in mountain	2n = 16 (6)	Choi & Jang TH -05
6	Gunjasan Mt., Goesan-gun, Chungbuk, KOREA	36°44'55.8"N, 127°52'44.6"E	927	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -06
7	Banyabong, Jirisan Mt., Jeonbuk, KOREA	35°18'45.5"N, 127°34'20.2"E	1,630	Rocky slope in mountain	2n = 16 (4)	Choi & Jang TH -07
8	Daeamsan Mt., Inje-gun, Gangwon, KOREA	38°13'10.8"N, 128°07'03.1"E	1,263	Forest in mountain	2n = 16 (2)	Choi & Jang TH -08
9	Baegunsan Mt., Gwangyang-si, Jeonnam, KOREA	35°06'23.1"N, 127°37'17.8"E	1,169	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -09
10	Daechongbong, Seoraksan Mt., Gangwon, KOREA	38°07'06.9"N, 128°27'55.0"E	1,685	Forest in mountain	2n = 16 (5)	Choi & Jang TH -10
11	Kkeutcheong, Seoraksan Mt., Gangwon, KOREA	38°07'17.1"N, 128°27'33.4"E	1,625	Forest in mountain	2n = 16 (6)	Choi & Jang TH -11
12	Chiaksan Mt., Wonju-si, Gangwon, KOREA	37°21'59.6"N, 128°03'14.4"E	1,134	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -12
13	Bukgachi, Sangju-si, Gyeongbuk, KOREA	36°34'07.0"N, 127°49'35.6"E	725	Forest in mountain	2n = 16 (5)	Choi & Jang TH -13
14	Jeongbyeongsan Mt., Changwon-si, Gyeongnam, KOREA	35°15'03.0"N, 128°42'13.4"E	398	Forest in mountain	2n = 16 (2)	Choi & Jang TH -14
15	Hongdo-ri, Sinan-gun, Jeonnam, KOREA	34°41'25.9"N, 125°12'12.3"E	256	Forest in mountain	2n = 16 (5)	Choi & Jang TH -15
16	Sineosan Mt., Gimhae-si, Gyeongnam, KOREA	36°09'45.3"N, 128°57'03.1"E	546	Forest in mountain	2n = 32 (5)	Choi & Jang TH -16
17	Jangboksan Mt., Changwon-si, Gyeongnam, KOREA	35°10'22.8"N, 128°40'46.6"E	562	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -17
18	Gasam Mt., Chilgok-gun, Gyeongbuk, KOREA	36°02'11.4"N, 128°34'40.6"E	878	Forest in mountain	2n = 32 (5)	Choi & Jang TH -18
19	Mungapdo, Ongjin-gun, Gyeonggi, KOREA	37°10'52.9"N, 126°05'51.4"E	160	Grassland in mountain	2n = 16 (5)	Choi & Jang TH -19
20	Manisan Mt., Ganghwado, Gyeonggi, KOREA	37°36'55.9"N, 126°25'47.2"E	439	Rocky slope in mountain	2n = 16 (5)	Choi & Jang TH -20
21	Misiryong, Inje-gun, Gangwon, KOREA	38°14'55.0"N, 128°24'38.8"E	591	Forest in mountain	2n = 16 (5)	H. J. Choi et al. 010017
<i>A. thunbergii</i> var. <i>deltoides</i>						
22	Gayasan Mt., Hapcheon-gun, Gyeongnam, KOREA	35°49'25.1"N, 128°07'13.5"E	1,390	Rocky slope in mountain	2n = 16 (3)	Choi & Jang THd -01

Table 1. Continued.

Pop	Locality	Geographical position	Elev. (m)	Habitat	CN (NI)	Voucher
<i>A. thunbergii</i> var. <i>teretifolium</i>						
23	Hyangjeokbong, Deogyusan Mt., Jeonbuk, KOREA	35°51'34.3"N, 127°44'46.3"E	1,613	Rocky slope in mountain	2n = 16 (5)	Choi & Jang THt-01
24	Namdeogyusan Mt., Geochang-gun, Gyeongnam, KOREA	35°46'16.8"N, 127°40'14.8"E	1,444	Rocky slope in mountain	2n = 16 (5)	Choi & Jang THt-02
25	Sseoribong, Jirisan Mt., Gyeongnam, KOREA	35°19'59.7"N, 127°43'57.5"E	1,659	Rocky slope in mountain	2n = 16 (5)	Choi & Jang THt-03
26	Cheonwangbong, Jirisan Mt., Gyeongnam, KOREA	35°51'40.8"N, 127°44'44.2"E	1,563	Rocky slope in mountain	2n = 16 (5)	Choi & Jang THt-04
<i>A. sacculiferum</i>						
27	Gwangeum-ri, Andong-si, Gyeongbuk, KOREA	36°30'08.3"N, 128°40'46.2"E	121	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-01
28	Gallasan Mt., Andong-si, Gyeongbuk, KOREA	36°29'43.9"N, 128°45'19.4"E	232	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-02
29	Deongnimsan, Yeonggwang-gun, Jeonnam, KOREA	35°24'24.2"N, 126°29'09.2"E	117	Forest in mountain	2n = 32 (5)	Choi & Jang SA-03
30	Seokgok-ri, Changwon-si, Gyeongnam, KOREA	35°06'32.1"N, 128°32'21.2"E	4	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-04
31	Gyo-dong, Yeosu-si, Gyeonggi, KOREA	37°16'38.5"N, 127°38'28.5"E	73	Forest margin and lowland pasture	2n = 16 (5)	Choi & Jang SA-05
32	Bamtijae, Cheongdo-gun, Gyeongbuk, KOREA	35°36'13.7"N, 128°41'35.0"E	530	Forest margin and lowland pasture	2n = 16 (5)	Choi & Jang SA-06
33	Mulhan-ri, Yeongdong-gun, Chungbuk, KOREA	36°07'29.4"N, 127°53'63.7"E	268	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-07
34	Taegisan Mt., Hoengseong-gun, Gangwon, KOREA	37°35'56.5"N, 128°18'30.0"E	745	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-08
35	Segeolsan Mt., Namwon-si, Jeonbuk, KOREA	35°24'10.7"N, 127°32'06.5"E	560	Forest margin and lowland pasture	2n = 16 (5)	Choi & Jang SA-09
36	Bulmosan Mt., Changwon-si, Gyeongnam, KOREA	35°10'01.9"N, 128°44'36.3"E	760	Forest in mountain	2n = 16 (5)	Choi & Jang SA-10
37	Hyodong-ri, Gyeongju-si, Gyeongbuk, KOREA	35°43'49.8"N, 129°22'59.4"E	320	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-11
38	Erdaobaihe, Jilin, CHINA	42°27'30.3"N, 128°09'20.5"E	660	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-12
39	Sikhote-Alin, Primorsky, RUSSIA	44°48'09.5"N, 136°19'29.6"E	2	Forest margin and lowland pasture	2n = 32 (5)	Choi & Jang SA-13

lowland meadows, forest margins and at riversides, while *A. thunbergii* usually occurs in rather different habitat types, such as sunny slopes of the rocky mountain and the forest in mountainous areas (Fig. 4). Out of 26 populations of *A. thunbergii*, nine diploids and two tetraploids occurred mountain forest (42.3%), 14 diploid populations occurred in the mountain rocky slopes (53.8%), and one diploid population was found in mountain grassland (3.8%) (Fig. 4). Whereas, out of 13 populations, one diploid and one tetraploid of *A. sacculiferum* grew in the forest in mountain (15.4%), and three diploids and

eight tetraploids tend to occupy only in a forest margin and lowland pasture (84.6%) (Fig. 4).

Discussion

We observed two different ploidy levels (diploid and tetraploid) in both tentatively morphologically identified *A. thunbergii* and *A. sacculiferum* in various habitat conditions (Table 1). Compared to Choi and Oh (2011), tetraploids of *A. thunbergii* (Pop 16 and 18 in Table 1) were newly recorded

Fig. 2. Distribution of cytotypes of *Allium thunbergii* and *A. sacculiferum* in South Korea. Details of each population are given in Table 1.

in Korea. The main cytotype of *A. thunbergii* was diploid (92.3% of total). In contrast, the majority of *A. sacculiferum* was tetraploid (69.2% of total). Any population of the studied taxa did not harbor both cytotypes. In general, occurrence of triploids in sympatric $2x + 4x$ populations gives evidence of hybridization between diploid and tetraploid individuals while triploid detection in diploid populations indicates frequent production of unreduced gametes by diploids (Sanders, 1987; Noda and Kawano, 1998; Ramsey and Schemske, 1998). Absence of triploid record in our survey might be caused by either just extensive study with low number of analyzed plants

or low probability of triploid production in diploids. However, from the representative karyotypes (Ko et al., 2009) (Fig. 1), we can present a possibility that the tetraploids of *A. sacculiferum* might be originated via autopolyploidization of diploid *A. thunbergii* or *A. sacculiferum*. Forthcoming studies by molecular cytogenetics as well as molecular sequencing data are required to more accurately identify the parental diploids genomes of the tetraploids.

Allium thunbergii was more often found at higher elevations than *A. sacculiferum*, and exhibited a tendency to occur more frequently in rocky slopes and in forests of mountainous areas.

Fig. 3. Box-plot of elevational distribution of diploids ($2n = 16$) and tetraploids ($2n = 32$) of *Allium thunbergii* and *A. sacculiferum*.

On the other hand, *A. sacculiferum* clearly preferred forest margins and lowland pastures (Choi and Oh, 2011) (Figs. 3, 4, Table 1). The cytotypes differed with respect to elevation; the diploids were found more frequently at higher elevations than the tetraploids (Fig. 3). It has been believed in past that cytotypes of higher ploidy level occur more frequently in higher elevations and/or are better colonizers of once glaciated areas (Lewis, 1980; Bierzychudek, 1985) due to their better physiological and ecological flexibility to adapt to harsh conditions. Concerning genus *Allium*, several studies examined the correlation of cytotypes with elevation in various species. For example, tetraploids of *A. przewalskianum* Regel occur mainly in higher elevations than diploids in the Qinghai-Tibetan Plateau and adjacent regions of China (Xie-Kui et al., 2008), and tetraploids of *A. oleraceum* L. mainly occur in higher elevations than the penta- and hexaploid individuals in Slovakia (Šafářová et al., 2011). However, Duchoslav et al. (2010) reported that there was no difference in elevation among ploidy levels of *A. oleraceum* in neighbouring region (the Czech Republic). This could be explained by different origins of tetraploids in each region and/or differences in migration routes of each cytotype even in geographically close regions. Elevational segregation of cytotypes with diploids occurring in higher elevations than tetraploids has previously been reported for *Anthoxanthum* spp. (Felber-Girard et al., 1996; Filipová and Krahulec, 2006), *Chamerion angustifolium* (L.) Holub (Husband and Schemske, 1998) and *Senecio carniolicus*

Fig. 4. Cytotype distribution (%) of *Allium thunbergii* and *A. sacculiferum* in common habitats. Number of population observed are given in parentheses.

Willd. (Schönschwetter et al., 2007). Based on the results of this study, we can make a hypothesis on the phylogeography of Korean *A. thunbergii* complex (including *A. sacculiferum*) that firstly the diploid ancestor had settled along the higher elevational mountainous areas in natural habitats, and after polyploid formation tetraploids colonized various semi-natural habitats especially in lower elevational areas. However, additional wider range studies covering whole distribution range of *A. thunbergii* complex are required for the clear elucidation of its phylogeography in Korea.

Allium sacculiferum differs from *A. thunbergii* by minute morphology characters: length of the leaf and scape, cross section of leaf blades; *A. sacculiferum* has a clearly elongated leaf sheath, which is exposed above ground and a longer leaf and scape (Choi and Oh, 2011). In addition to the morphological identification, Ko et al. (2009) and Choi and Oh (2011) found that these closely related species are either diploid (*A. thunbergii*) or tetraploid (*A. sacculiferum*). Choi and Oh (2011) also noticed a diploid population that, based on morphological observations, belongs within the range of tetraploid *A. sacculiferum*. The morphological differentiation between tetraploids and diploids in several polyploid plant complexes is documented by several studies. Typically, polyploidy increases the sizes of cells (e.g., guard cells) and organs, and it can result in increase of more complex traits as well as overall habit (Müntzing, 1936; Otto and Whitton, 2000;

Levin, 2002; Beaulieu et al., 2008). For instance, the study of Sugiyama (2005) on *Lolium perenne* L. and *L. multiflorum* Lam. and Gao et al. (2016) on *Chrysanthemum lavandulifolium* (Fisch. ex Trautv.) Makino showed that leaves and stem (length, width) of tetraploids were bigger than those of diploids. Larger size of several traits in tetraploid *A. sacculiferum* in contrast to diploid *A. thunbergii* is consistent with the mentioned studies, and we hypothesize that the autotetraploid population of *A. thunbergii* showing larger size has been therefore recognized as *A. sacculiferum*. However, the diploids of *A. sacculiferum* (Pop 31, 32, 35 and 36 in Table 1) clearly had also elongated leaf sheath in four populations while some tetraploids (Pop 16 and 18 in Table 1) of *A. thunbergii* had a non-elongated to intermediate leaf sheath (H. J. Choi, pers. obs.). This could be explained by the adaptation of the respective population to various environmental factors. Therefore, the morphological characteristics of *A. thunbergii* and *A. sacculiferum* might be influenced by local adaption and polyploidization.

Although we studied populations that were morphologically identified as *A. thunbergii* and *A. sacculiferum* only in restricted part of the potential distribution area of these species, result from this study and additional biosystematics data suggest that *A. thunbergii* and *A. sacculiferum* do not still fulfil any species concept: (1) they are incompletely differentiated by morphological characters (morphological species concept), and overlap in majority of morphological characteristics including reproductive organs in particular (Choi and Oh, 2011; H. J. Choi, pers. obs.); (2) habitat differentiation (ecological species concept) is more or less incomplete (Fig. 4); (3) incomplete reproductive isolation (biological species concept) was recognized by artificially making viable seeds crossing diploid *A. thunbergii* and diploid *A. sacculiferum* (Yu et al., 1981b); and (4) Choi et al. (2012) showed an incomplete monophyletic network of DNA sequences (phylogenetic species concept) between *A. thunbergii* and *A. sacculiferum*.

Plants with different chromosome numbers are not infrequently found within the bounds of one taxonomic species (Friesen, 1992; Rice et al., 2015). The problem of the taxonomic rank of the members of one polyploidy series of plants is so far disputable. Based on Friesen (1992), we took up the following position in understanding the species range in the genus *Allium*. In the cases when definite populations of plants are the members of the polyploidy series, and besides they constantly differ in morphological characters and have the habitat of their own, we can distinguish them as independent taxonomic species. Friesen (1992) also suggested that it is untimely to impart the rank of a taxonomic species to cytotypes

Fig. 5. The holotype of *Allium thunbergii* G. Don.

with difference in the chromosome numbers but without revealing appreciable morphological differences. Consequently, *A. thunbergii* and *A. sacculiferum* should be regarded as a same species. Therefore, we propose to treat *A. sacculiferum* as an additional synonym of *A. thunbergii*. Additionally, *Allium thunbergii* G. Don var. *deltoides* (S. Yu. W. Lee & S. Lee) H. J. Choi & B. U. Oh is unified into the basic species of *A. thunbergii* var. *thunbergii*. Future studies involving various cases on the cytotype diversity and its taxonomic significance will provide a sound basis to understand more clearly the species boundary of *Allium*.

Taxonomic Treatment

Allium thunbergii G. Don., Mem. Wern. Soc. 6: 84, 1827.—
TYPE: Japan. UPS 8065 (holotype: UPS!, Fig. 5).

A. odorum Thunb., Fl. Jap 132, 1784. Non L. Type not traced.

A. morrisonense Hayata., Icon. Pl. Formos. 6. Suppl: 84,

1917; *A. bakeri* Regel var. *morrisonense* (Hayata) T. S. Lui & S. S. Yung., Fl. Taiwan. 5: 45, 1978. Type not traced.

A. sacculiferum Maxim., Prim. Fl. Amur. 281, 1859.—TYPE: Russia. On south Amur, one Dai travel over the Chinganskoi Piket, 21 Aug 1856, *K. I. Maximowicz. s. n.* (lectotype: LE!, designated by N. Friesen, 8 Aug 1996, photo CBU!).

A. ophiopogon H. Lév., Repert. Spec. Nov. Regni Veg. 12: 184, 1913.—TYPE: Korea. Quelpaert, in Parva insula Mouseum, in herbis, 9 Aug 1911, *Taquet 5213* (lectotype: TI!).

A. stenodon Nakai & Kitag., Rep. 1st. Sci. Exp. Manch. 4: 18, 1934.—TYPE: Korea. In monte Wu-ling-shan, 2 Sep 1933, *N. H. K. s. n.* (holotype: TI?).

A. komarovianum Vved., Bull. Univ. As. Centr. 19: 119, 1934.—TYPE: Russia. By Village Faddeevka on the river Suifun, 11 Sep 1931, *V. L. Komaov s. n.* (holotype: LE!, photo: CBU!).

A. sacculiferum Maxim. var. *viviparum* Sakata, Bull. Nat. Sci. Mus. Tokyo 7: 16, 1938.—TYPE: Japan. Keiki, pede Reikistan, Suigen, 6 Oct 1935, *T. Sakata s. n.* (holotype: TI!).

A. deltoidefistulosum S. Yu, W. Lee & S. Lee, Korean J. Pl. Taxon. 11: 30, 1981.—TYPE: Korea. Jeonbuk: Namwon, wet open grassland around pine forest at 600 m c. 800 m toward Segeolsan of Gonggan, *Yoo 6012* (lectotype: KWNU!; isolectotypes: KWNU!, JNU?, SNU?).

A. yuchuanii Y. Z. Zhao & J. Y. Chao, Acta Sci. Nat. Univ. Intramongolicae 20: 241, 1989. Type not traced.

A. cyaneum Regel var. *deltoides* S. Yu, W. Lee & S. Lee, Korean J. Pl. Taxon. 11: 29, 1981.—TYPE: Korea. Gyeongnam: Gayasan, grassland and soil on the rocks c. 1,300–1,400 m, along the trail to the peak, *Yoo 5920* (holotype: Wonkwang University herbarium; isotypes: KWNU!, JNU, SNU); *A. thunbergii* G. Don var. *deltoides* (S. Yu., W. Lee & S. Lee) H. J. Choi & B. U. Oh, Korean J. Pl. Taxon. 33: 351, 2003.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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