

A new distribution record of *Chrysosplenium grayanum* Maxim. (Saxifragaceae) in Korea: Evidence from morphological and molecular data

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Chrysosplenium grayanum Maxim. (Series *Nepalensia*), which had been known to be restricted to Japan, was newly discovered from Mt. Cheongtae in Yeonggwang-gun, Jeollanam-do, located in the southern part of the Korean Peninsula. Species identification was confirmed using morphological characteristics and DNA sequence data, while comparing with materials obtained from Japan and herbarium specimens. *Chrysosplenium grayanum* is clearly distinguished from the remaining taxa of the genus *Chrysosplenium* by having glabrous plant body, opposite leaves, cylindrical papillae with roundish head at the tip on the smooth seed surface, and four stamens. Molecular sequence data of the nuclear ribosomal ITS regions, chloroplast *rbcL* and *matK* genes strongly supported that this previously unknown *Chrysosplenium* species from Korea is *C. grayanum*. Taking the molecular and the morphological evidence into consideration, it is clear that newly discovered *Chrysosplenium* population in Korea is conspecific with the widely distributed *C. grayanum* in Japan. In this paper, we provide a description, illustration, and photo images of *Chrysosplenium grayanum* from Korea and also a key to the *Chrysosplenium* species in Korea.

Keywords: *Chrysosplenium grayanum*, *Chrysosplenium*, Saxifragaceae, nrITS, cp *rbcL* and *matK* genes

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INTRODUCTION

Chrysosplenium L. (Saxifragaceae) is composed of approximately 60 species (Hara, 1957), and usually defined within the Saxifragaceae by having tetramerous flowers without petals (Bensel and Palser, 1975; Kim, 2007; Kim *et al.*, 2018). *Chrysosplenium* species are primarily distributed in temperate regions of the Northern Hemisphere, except for two disjunct species in South America; *C. valdevicum* Hook. and *C. macranthum* Hook. (Hara, 1957; Spongberg, 1972; Ye and Zhang, 1994; Wakabayashi and Takahashi, 1999; Han *et al.*, 2011). In the Northern Hemisphere, Eastern Asia (including Sino-Himalayan regions, China, Korea and Japan) is well known as the center of diversity for the genus *Chrysosplenium* (Hara, 1957; Spongberg, 1972; Chung and Kim, 1988). Hara (1957), in his synopsis of *Chrysosplenium*, recognized 17 series based on detailed morphological studies,

of which nine species representing five series are distributed in Korea (Chung and Kim, 1988; Han *et al.*, 2011; Kim and Kim, 2011; 2015). However, the number of taxa in Korea is still controversial due to extreme morphological variation in their diagnostic characters and seasonal changes in the morphology of vegetative parts (Nakai, 1952; Hara, 1957; Chung, 1965; Park, 1974; Kim, 2007; Lee, 1980).

Chrysosplenium grayanum Maxim. belongs to the series *Nepalensia* Maxim., and is known as an endemic species of Japan. The species is widely distributed in all Japanese Islands (Wakabayashi, 2001). *Chrysosplenium grayanum* was first described by Maximowicz (1877) from Hokkaido, Japan mainly on the basis of plant habit, the number of stamens, and papillae shapes on the seed surfaces. In the original description, Maximowicz (1877) stated that *C. grayanum* is very distinct from other species in the genus in having four stamens and seed

papillae with a globose, rough head. Meanwhile, Nakai (1914), in his floristic research on Jeju Island, reported that *C. grayanum* occurred on Mt. Halla in Korea without voucher specimens and morphological descriptions.

Since Nakai (1914) reported *C. grayanum* in Korea, it has been accepted as a Korean indigenous species by most Korean botanists (Lee and Oh, 1985; Lee, 1996; Kim and Kim, 1997; Lee, 2006). However, Hara (1957) recognized *C. grayanum* as being endemic to Japan, pointing out the possibility of misidentification between *C. grayanum* and *C. pseudofauriei* H. Lév.

However, *Chrysosplenium* species are quite often misidentified because they are extremely variable in leaf morphology depending on growing period and habitat difference (Hara, 1957; Kim, 2007; Kim and Kim, 2011). In addition, the taxonomic difficulty has been attributed to few diagnostic characters and differences in appearance (e.g., flowers and capsules, color of bracteal leaves, shape of sterile branches after fruiting) between living plants and dried herbarium specimens (Hara, 1957; Kim, 2007). For these reasons, *C. grayanum* was still considered by some Korean botanists to be distributed throughout the Korean Peninsula, including Hamgyeongbuk-do, Gyeongsangbuk-do, and Jeju-do (Lee and Oh, 1985; Lee, 1996; Kim and Kim, 1997; Lee, 2006). However, other Korean botanists (Park, 1974; Chung and Kim, 1988) disputed the presence of *C. grayanum* in Korea, supporting Hara's reviews. In particular, Chung and Kim (1988) demonstrated that *C. grayanum* was not distributed in Korea after they did not find this species in their targeted *C. grayanum* survey of the Korean Peninsula.

During recent field works in Mt. Cheongtae, located in Yeonggwang-gun, Jeollanam-do, Korea in March of 2017, the second and third authors recognized a *Chrysosplenium* species with similar morphological characters with *C. grayanum* Maxim. in the series *Nepalensia*. After comprehensive morphological examination of specimens and literature (Maxim, 1877; Chung and Kim, 1988; Wakabayashi, 1999), we concluded that this *Chrysosplenium* species is *C. grayanum* Maxim. Sequence data from three molecular markers [ITS regions of nuclear ribosomal DNA (nrITS), chloroplast (cp) *rbcL*, and *matK*] of *C. grayanum* of Japan and Korea were also consistent with the morphology. Therefore, we confirm the distribution of *C. grayanum* in Korea based on the evidence from morphological and molecular data, and constructed revised key to identify Korean *Chrysosplenium* species based on specimens collected from the survey.

MATERIALS AND METHODS

The newly discovered *C. grayanum* from Mt. Cheong-

tae was exhaustively compared against the type specimens [Japan, Hakodate, 1861, *Maximowicz s.n.* (holotype, P!, MNHN-P-00709321, available at <http://science.mnhn.fr/>; isotype, K!, K000732800, available at <https://apps.kew.org/>), and with the original description of Maximowicz (1877). In addition, the new materials from Korea were compared with *C. grayanum* collected from Hokkaido and Honshu in Japan (Table 1). Morphological characters were studied using stereo microscope (ZX7, Olympus, Japan) and upright metallurgical microscope (BX51, Olympus, Japan). The seeds were observed with a Scanning Electron Microscope (JSM-6390, JEOL, Japan) after coating the completely dried seed surface with gold (Sputter Coater 108auto, Cressington, UK).

For molecular identification of the new materials, we obtained nrITS, cp *rbcL* and *matK* sequence data for total 30 individuals representing 10 species of *Chrysosplenium* from Korea and Japan (nine in Korea and two in Japan). Among them, two outgroup taxa [*C. flagelliferum* F. Schmidt and *C. japonicum* (Maxim.) Makino] were selected based on the results of a previous phylogenetic analysis (Nakazawa *et al.*, 1997). All voucher specimens were deposited at the herbarium of National Institute of Biological Resources (KB). Total genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Germany). The nrITS region was amplified and sequenced with a modified ITS1 primer by Kim and Kim (2011) and ITS4 (White *et al.*, 1990), *rbcL* region with 1F and 724R (Fay *et al.*, 1997), and *matK* region with 3F_Kim_r and 1R_Kim_f (K. J. Kim, per. comm.). Each PCR reaction was carried out according to procedures described in previous studies (Nakazawa *et al.*, 1997; Kim and Kim, 2011). Sequence data were assembled and edited using Sequencher version 5.1 (Gene Codes Co., USA), aligned with ClustalW (Thompson *et al.*, 1994), and proofread by eye in BioEdit version 7.2.5 (<http://www.mnion.ncsu.edu/BioEdit/biodeit.html>). Phylogenetic analyses were performed using maximum parsimony (MP) and neighbor-joining (NJ) methods. The MP and NJ analysis were implemented in PAUP* 4.0b10 (Swofford, 2002). Heuristic MP analysis was replicated 1,000 times with random stepwise addition, tree bisection-reconnection (TBR) branch swapping, and saving multiple trees. The MP bootstrap analyses (Felsenstein, 1985) were performed using 1,000 replicates with TBR branch swapping and a random addition sequence. The combined dataset of nrITS and two cp DNA regions were evaluated using incongruence length difference (ILD) test in PAUP* 4.0b10 (Swofford, 2002) under the partition homogeneity test. The Kimura's 2-parameter model (Kimura, 1980) was applied in the NJ analysis. Bootstrap analysis was performed 1,000 times to evaluate reliability for each factor.

Table 1. List of taxa, voucher specimens, GenBank accession numbers and WIGIS numbers of sequences newly generated during this study. WIGIS is Wildlife Integrated Genetic Information System in National Institute of Biological Resources, Korea.

Taxon	Voucher	GenBank / WIGIS number (ITS, <i>rbcL</i> , <i>matK</i>)
<i>Chrysosplenium aureobracteatum</i>	Korea, Gangwon-do, Mt. Gwangdeog, <i>LeeJD et al. 17127-1</i> (NIBRVP0000621581)	MK989508, MK989529, MK989559/ WBN0339300, WBN0337344, WBN0339146
	Korea, Gangwon-do, Mt. Gwangdeog, <i>LeeJD et al. 17127-2</i> (NIBRVP0000621582)	MK989509, MK989532, MK989562/ WBN0339301, WBN0337345, WBN0339147
<i>C. barbatum</i>	Korea, Jeollanam-do, Woldeung-myeon, <i>LeeJD et al. 17008-1</i> (NIBRVP0000611575)	MK989505, MK989530, MK989560/ WBN0339234, WBN0337278, WBN0339080
	Korea, Gyeongsangbuk-do, Mt. Danseok, <i>LeeJD et al. 17020-1</i> (NIBRVP0000611604)	MK989506, MK989534, MK989564/ WBN0339244, WBN0337288, WBN0339090
<i>C. flagelliferum</i>	Korea, Gangwon-do, Mt. Gwangdeog, <i>LeeJD et al. 17066</i> (NIBRVP0000611792)	MK989507, MK989531, MK989561/ WBN0339287, WBN0337331, WBN0339133
	Korea, Gyeonggi-do, Mt. Cheonma, <i>LeeJD et al. 17014</i> (NIBRVP0000611591)	MK989499, MK989555, MK989585/ WBN0339242, WBN0337286, WBN0339088
	Korea, Gangwon-do, Mt. Cheongtae, <i>LeeJD et al. 17052-1</i> (NIBRVP0000611766)	MK989500, MK989553, MK989583/ WBN0339269, WBN0337313, WBN0339115
<i>C. flaviflorum</i>	Korea, Gyeongsangbuk-do, Ulleung-do, <i>LeeJD et al. 17122</i> (NIBRVP0000616561)	MK989501, MK989554, MK989584/ WBN0339302, WBN0337346, WBN0339148
	Korea, Chungcheongbuk-do, Mt. Gyemyeong, <i>LeeJD et al. 17030</i> (NIBRVP0000611623)	MK989513, MK989539, MK989569/ WBN0339253, WBN0337297, WBN0339099
	Korea, Gyeongsangbuk-do, Mt. Cheonglyang, <i>LeeJD et al. 17039</i> (NIBRVP0000611745)	MK989514, MK989537, MK989567/ WBN0339257, WBN0337301, WBN0339103
<i>C. grayanum</i>	Korea, Gangwon-do, Mt. Chiak, <i>LeeJD et al. 17048</i> (NIBRVP0000611758)	MK989515, MK989538, MK989568/ WBN0339263, WBN0337307, WBN0339109
	Japan, Hokkaido, Sapporo, Mt. Maruyama, <i>Nakamura 16401</i> (NIBRVP0000647409)	MK989524, MK989544, MK989574/ WBN0339364, WBN0339030, WBN0339222
	Japan, Hokkaido, Sapporo, Mt. Maruyama, <i>Nakamura 16402</i> (NIBRVP0000647418)	MK989523, MK989545, MK989575/ WBN0339365, WBN0339031, WBN0339223
<i>C. japonicum</i>	Japan, Hyogo prefecture, Sasayama, <i>Lee JH & JS Shin s. n.</i> (NIBRVP0000709264)	MK989525, MK989546, MK989576/ WBN0346807, WBN0350456, WBN0350457
	Korea, Jeollanam-do, Mt. Cheongtae, <i>LeeJD et al. 17090-1</i> (NIBRVP0000611822)	MK989522, MK989549, MK989579/ WBN0339295, WBN0337339, WBN0339141
	Korea, Jeollanam-do, Mt. Cheongtae, <i>LeeJD et al. 17090-2</i> (NIBRVP0000611823)	MK989520, MK989548, MK989578/ WBN0339296, WBN0337340, WBN0339142
	Korea, Jeollanam-do, Mt. Cheongtae, <i>LeeJD et al. 17090-3</i> (NIBRVP0000611824)	MK989521, MK989547, MK989577/ WBN0339297, WBN0337341, WBN0339143
	Korea, Jeju-do, Gwetmuloreum, <i>LeeJD et al. 17003-1</i> (NIBRVP0000611566)	MK989503, MK989558, MK989588/ WBN0339228, WBN0337272, WBN0339074
<i>C. kamtschaticum</i>	Korea, Jeollabuk-do, Mt. Chaegye, <i>LeeJD et al. 17022</i> (NIBRVP0000611607)	MK989502, MK989556, MK989586/ WBN0339246, WBN0337290, WBN0339092
	Korea, Chungcheongnam-do, Palbong-myeon, <i>LeeJD et al. 17025-1</i> (NIBRVP0000611612)	MK989504, MK989557, MK989587/ WBN0339251, WBN0337295, WBN0339097
	Japan, Hokkaido, Sapporo, Mt. Maruyama, <i>Nakamura 16403</i> (NIBRVP0000647509)	MK989516, MK989536, MK989566/ WBN0346803, WBN0346805, WBN0346804
<i>C. pseudofauriei</i>	Korea, Jeju-do, Haean-dong, <i>LeeJD et al. 17043</i> (NIBRVP0000611753)	MK989528, MK989552, MK989582/ WBN0339258, WBN0337302, WBN0339104
	Korea, Gangwon-do, Mt. Cheongtae, <i>LeeJD et al. 17051-1</i> (NIBRVP0000611764)	MK989526, MK989550, MK989580/ WBN0339267, WBN0337311, WBN0339113
	Korea, Chungcheongbuk-do, Gagok-myeon, <i>LeeJD et al. 17086</i> (NIBRVP0000611817)	MK989527, MK989551, MK989581/ WBN0339291, WBN0337335, WBN0339137
<i>C. ramosum</i>	Korea, Chungcheongbuk-do, Daegang-myeon, <i>LeeJD et al. 17097-1</i> (NIBRVP0000614442)	MK989517, MK989541, MK989571/ WBN0339298, WBN0337342, WBN0339144
	Korea, Gangwon-do, Mt. Taegi, <i>LeeJD et al. 17147</i> (NIBRVP0000616611)	MK989518, MK989543, MK989573/ WBN0339303, WBN0337347, WBN0339149
	Korea, Gyeongsangbuk-do, Mt. Irwol, <i>LeeJD et al. 17205-1</i> (NIBRVP0000623390)	MK989519, MK989542, MK989572/ WBN0339307, WBN0346806, WBN0339153

Table 1. Continued.

Taxon	Voucher	GenBank / WIGIS number (ITS, <i>rbcL</i> , <i>matK</i>)
<i>C. valdepilosum</i>	Korea, Gangwon-do, Mt. Taegi, <i>LeeJD et al. 17053-1</i> (NIBRVP0000611768)	MK989512, MK989533, MK989563/ WBN0339271, WBN0337315, WBN0339117
	Korea, Jeollabuk-do, Mt. Jiri, <i>LeeJD et al. 17057</i> (NIBRVP0000611776)	MK989510, MK989535, MK989565/ WBN0339277, WBN0337321, WBN0339123

RESULTS AND DISCUSSION

Description

Chrysosplenium grayanum Maxim., Bull. Acad. Imp. Sci. Saint-Petersbourg xxiii, 348, 1877.

Similar to *C. pseudofauriei* but differing by usually having four to six stamens opposite the sepals (vs. eight stamens), smaller flowers 1.5–2.5 mm in diameter (vs. 3.0–4.0 mm in diameter), glabrous plant body (vs. glabrous plant body except petiole of rosette leaves), and cylindrical papillae with roundish head at the tip on smooth seed surfaces (vs. cylindrical papillae with truncate tip on scabrous seed surfaces).

Herbs, perennial, hermaphroditic, rhizomatous, glabrous. Rhizomes creeping, elongate. Roots fibrous, white, from lower nodes of branches. Sterile branches well developed, decumbent, simple or branched, 7–8 cm tall, ca. 1 mm in diam., fleshy, glabrous. Leaves on sterile branches not in rosette, simple, opposite, 2 or 3 pairs, estipulate, petiole; petioles 1–3 mm long; blade unlobed, ovate-orbicular, 4–6 × 5–8 mm, apex obtuse, base cuneate, margins obscurely undulate or crenate, with 3 or 4 teeth on each side. Flowering stems similar to sterile branches, (4–)11–20 cm tall. Leaves on flowering stems basal and cauline. Basal leaves on flowering stems 1 or 2 pairs, withered before flowering; petioles 6–8 mm; blade oblong-ovate, 1.4–1.8 × 1.2–1.5 mm, apex obtuse, base cuneate, margins crenate. Cauline leaves on flowering stems 2–5 (–7) pairs; petioles 3–5 mm; blades ovate-orbicular, (0.3–)0.6–1(–1.7) × (0.3–)0.5–0.8(–1.7) cm, apex rounded, base rounded to subtruncate, margins obscurely undulate or crenate, with 4–6 teeth on each side, adaxial surface green, abaxial surface light green. Inflorescences terminal, cymes, 9–12-flowered, surrounded by bracteal leaves; bracteal leaves several, compact; petiole of bracteal leaves up to 3 mm long; blade of bracteal leaves broadly ovate, 0.7–1.5 × 0.7–1.2 cm, margins crenate, with 3–6 teeth on each side, both surfaces green to yellowish-green during flowering. Flowers bisexual, 4-merous, campanulate, 1.5–2.5 mm in diam.; sepals free, persistent, 4, erect,

imbricate in bud, petaloid, yellowish-green, nearly orbicular, slightly saccate, 0.7–1.5 × ca. 0.5 mm, apex rounded; petals absent; stamens 4 (–6), episepalous; filaments filiform, ca. 0.8 mm long; anthers yellow, subglobose, 2-locular, dehiscent longitudinally; ovary subinferior, 1-locular, 2-carpellate; ovules many; styles 2, free, very short; stigmas 2, punctate; placentation parietal. Fruits capsules, 2-lobed, 4–5 × 6–8 mm; lobes ascending, dehiscent along an adaxial suture, horn-shaped, subequal, glabrous. Seeds brown to dark brown, usually 30–36, ovoid-ellipsoid, 0.5–0.7 × 0.4–0.5 mm, moderately papillose on smooth surfaces; papillae cylindrical with roundish head at the tip. Chromosome number $2n = 22$ (Wakabayashi, 2001).

Korean name: Gwaeng-i-nun 팽이눈

Phenology: Flowering in April to May, and fruiting from May to June.

Distribution: Japan, Korea (Yeonggwang-gun, Jeollanam-do)

Specimens examined: KOREA. Jeollanam-do: Yeonggwang-gun, Daema-myeon, Songjuk-ri, Mt. Cheongtae, ca. 278 m, 25 May 2017, *LeeJD et al. 17090-1* (KB, NIBRVP0000611822), *LeeJD et al. 17090-2* (KB, NIBRVP0000611823), *LeeJD et al. 17090-3* (KB, NIBRVP0000611824). JAPAN. Hokkaido, Chuo-ku, Sapporo city, Mt. Maruyama, 15 October 2017, *16401* (KB, NIBRVP0000647409), *16402* (KB, NIBRVP0000647418); Honshu, Hyogo Pref., Sayama city, elev. ca. 376 m, 29 April 2018, *Lee JH & JS Shin s. n.* (KB, NIBRVP0000709264).

Surveys of habitat status and distribution

During field research in 2017, conducted in the southeastern part of the Korean Peninsula, a new *C. grayanum* site was found in Korea. *Chrysosplenium grayanum* is very rare in distribution, and only a few isolated populations are found from Mt. Cheongtae, Yeonggwang-gun, Jeollanam-do. Each subpopulation of this species, which contains 50–300 individuals, is usually found in moist habitats with rich soils on forest floors and along streams at an elevation of ca. 278 m. The forest is commonly dominated by *Styrax japonicus* Siebold & Zucc., *Paulownia coreana* Uyeki, *Weigela subsessilis* (Nakai) L. H. Hailey, *Prunus sargentii* Rehder, *Aralia elata* (Miq.) Seem.,

Table 2. Statistics of nrITS, cp *rbcL*, *matK* regions, and cpDNA combined data set used in our phylogenetic analyses of *Chrysosplenium*.

	Nuclear ribosomal DNA		Chloroplast DNA	
	ITS	<i>rbcL</i>	<i>matK</i>	<i>rbcL</i> + <i>matK</i> Combined
No. of accessions	29	29	29	29
Sequence length (bp)	650–656	656	776–782	1432–1438
Aligned length (bp)	682	656	794	1450
G + C ratio (%)	44.4–50.4	43.0–43.9	31.2–32.2	36.9–37.4
No. of variable sites (%)	194 (28.4)	27 (4.1)	91 (11.5)	118 (8.1)
No. of parsimony-informative sites (%)	187 (27.4)	21 (3.2)	77 (9.7)	98 (6.8)
Intraspecific K2P distance (mean)*	0–0.0061 (0.0023)	0–0.0046 (0.0015)	0–0.0052 (0.0013)	0–0.0042 (0.0010)
Interspecific K2P distance (mean)*	0.0108–0.1377 (0.1584)	0–0.0248 (0.0138)	0–0.0492 (0.0358)	0–0.0372 (0.0249)
No. of MP trees	1	2	1	1
Tree length	285	37	114	153
Consistency index	0.853	0.757	0.868	0.830
Retention index	0.959	0.931	0.956	0.945

*Outgroup taxa excluded.

Akebia quinata (Houtt.) Decne., *Codonopsis lanceolata* (Siebold & Zucc.) Trautv. and is mixed with herbal species such as *Polygonum thunbergii* Siebold & Zucc. and *Carex forficula* Franch. & Sav. var. *forficula*.

Molecular data analysis

We obtained nrITS, cp *rbcL* and *matK* sequences from 29 accessions of 10 *Chrysosplenium* taxa (Table 1). The length of the nrITS region in *Chrysosplenium* was 650–656 bp. The final alignment of the nrITS region included 682 sites, of which 187 (27.4%) were parsimony informative. The length of cpDNA *rbcL* and *matK* regions were 656 and 776–782 bp, respectively. The combined *rbcL* and *matK* sequences were aligned with a consensus length of 1450 bp, of which 98 (6.8%) were parsimony informative. The incongruence length difference (ILD) test showed that the incongruity between nrITS region and two cpDNA regions (*rbcL* and *matK*) was just not significant at the 1% level ($p = 0.01$). Thus, to avoid combining different phylogenetic information, nrITS, *rbcL*, *matK*, and a combined cpDNA dataset were analyzed, respectively. The statistics of data and phylogenetic analyses for the MP tree and NJ tree are provided in Table 2.

The phylogenetic tree from MP and NJ methods produced similar topologies based on the combined cpDNA and nrITS data, respectively (tree not shown). Therefore the better resolved MP tree of the nrITS data is presented with bootstrap support values (Fig. 4). The MP analysis of the nrITS sequence data produced only one optimal tree (CI = 0.853, RI = 0.959), in which 10 *Chrysosplenium*

species in Korea and Japan were divided into seven series (BS = 58–100%, Fig. 4); *Nepalensia*, *Sinica* Maxim., *Flagellifera* Maxim., *Alternifolia* Maxim., *Pilosa* Maxim., *Kamtschatica* H. Hara, and *Oppositifolia* H. Hara that correspond well with Hara's (1957) species groups. Phylogenetic analysis showed that the six accessions of *C. grayanum* (three from Korea and three from Japan) nested together and formed a well-supported monophyletic group (BS = 100%) (Fig. 4). Compared to the sequences of Japanese individuals, the newly discovered Korean individuals differ by 2–5 bp in nrITS, 2 bp in *matK*, 1 bp in *rbcL*. However, these differences may be an indication of local differentiation between Korea and Japan. All accessions of *C. grayanum* consistently formed a monophyletic group in MP and NJ analyses of both nrITS and combined cp DNA sequences. In addition, the Korean individuals are indistinguishable from Japanese individuals of *C. grayanum* based on their diagnostic morphological characters.

Taking the molecular evidence and the morphology into consideration (Figs. 1–4), it is clear that newly discovered *Chrysosplenium* population in Korea is conspecific with the widely distributed *C. grayanum* in Japan. Our study presents morphological and molecular evidence that *C. grayanum* is distributed in Korea, which until recently was uncertain. On the other hand, The newly discovered *C. grayanum* population was found to be distributed only in a very limited site of Jeollanam-do, ca. 200 km from Jeju Island, the location of the first report by Nakai (1914). However, Chung and Kim (1988), failed to find *C. grayanum* populations in Mt. Halla and Mt. Juwang, where this

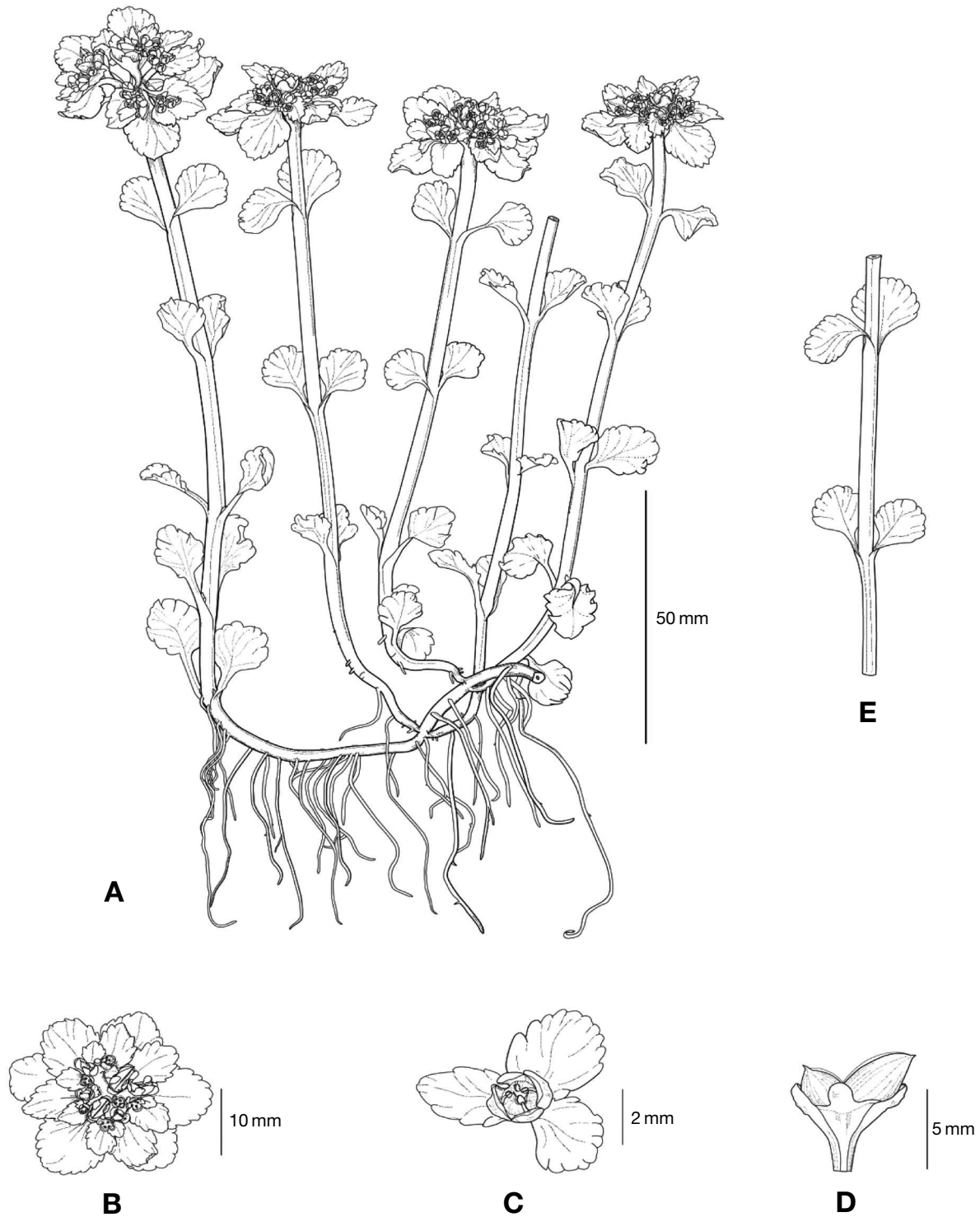


Fig. 1. Illustrations of *Chrysosplenium grayanum* Maxim. A. Flowering plant; B. Inflorescence; C. Sepals and stamens; D. Capsule with persistent sepals; E. Leaf arrangement. Illustrations of *Chrysosplenium grayanum* were drawn by Park Chan-Ae.

species was previously reported. If we take this into account, it can be considered that this is the first statement after 106 years since Nakai's statement.

To date, there are only three subpopulations record from Mt. Cheongtae, all of which are in non-protected areas in forests fragments and disturbed transitions zones.

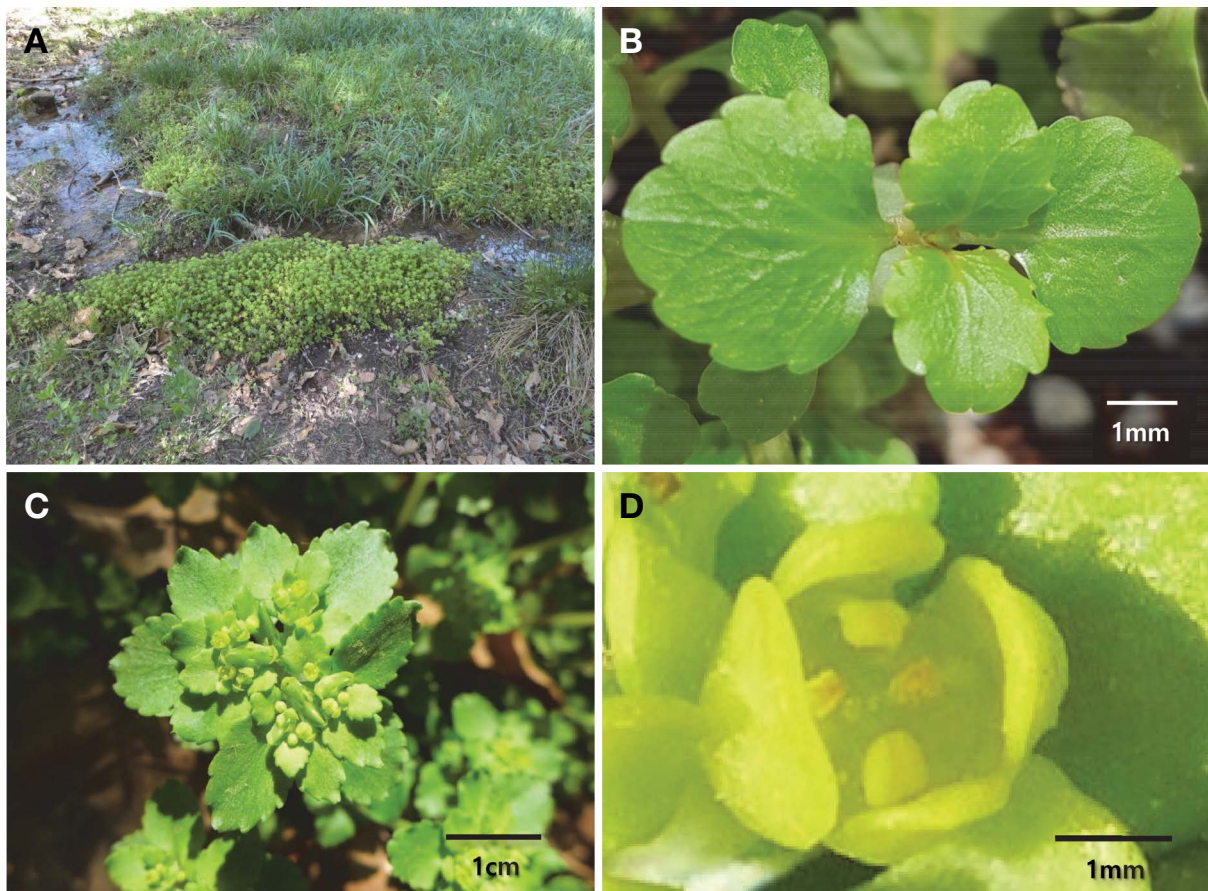


Fig. 2. Photos of *Chrysosplenium grayanum* Maxim. A. Plant habit during flowering; B. Leaves; C. Inflorescence with bract leaves; D. Close-up of sepals and stamens.

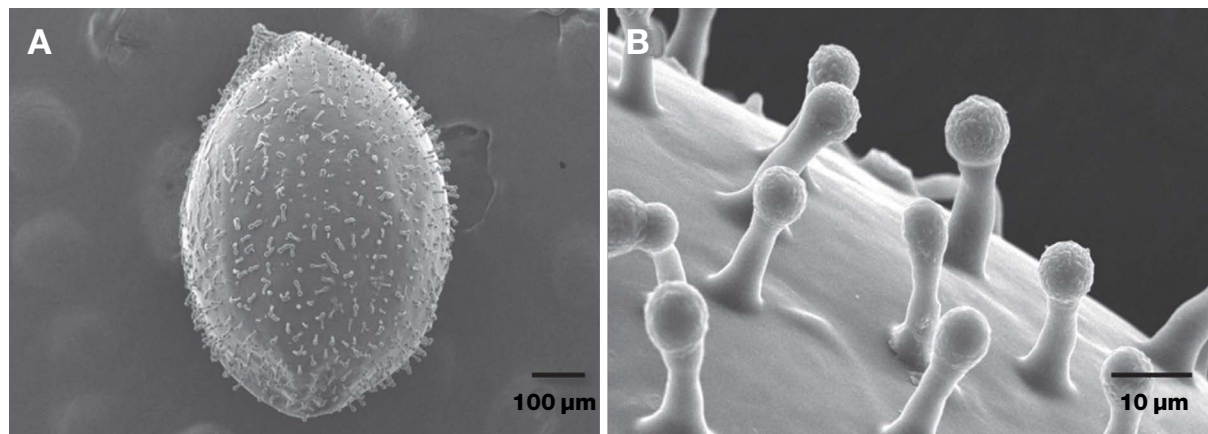


Fig. 3. Scanning electron micrograph of seed of *Chrysosplenium grayanum* Maxim. A. Seed; B. Close-up of seed surface, showing smooth surface with cylindrical papillae with roundish head at the tip.

Therefore, this species is listed as endangered (EN) in Korea (IUCN, 2019) due to its rarity and fragmentation of subpopulations. More field work is needed to check

for undetected populations in similar conditions, like in southern part of Korean Peninsula including islands along the southern coast.

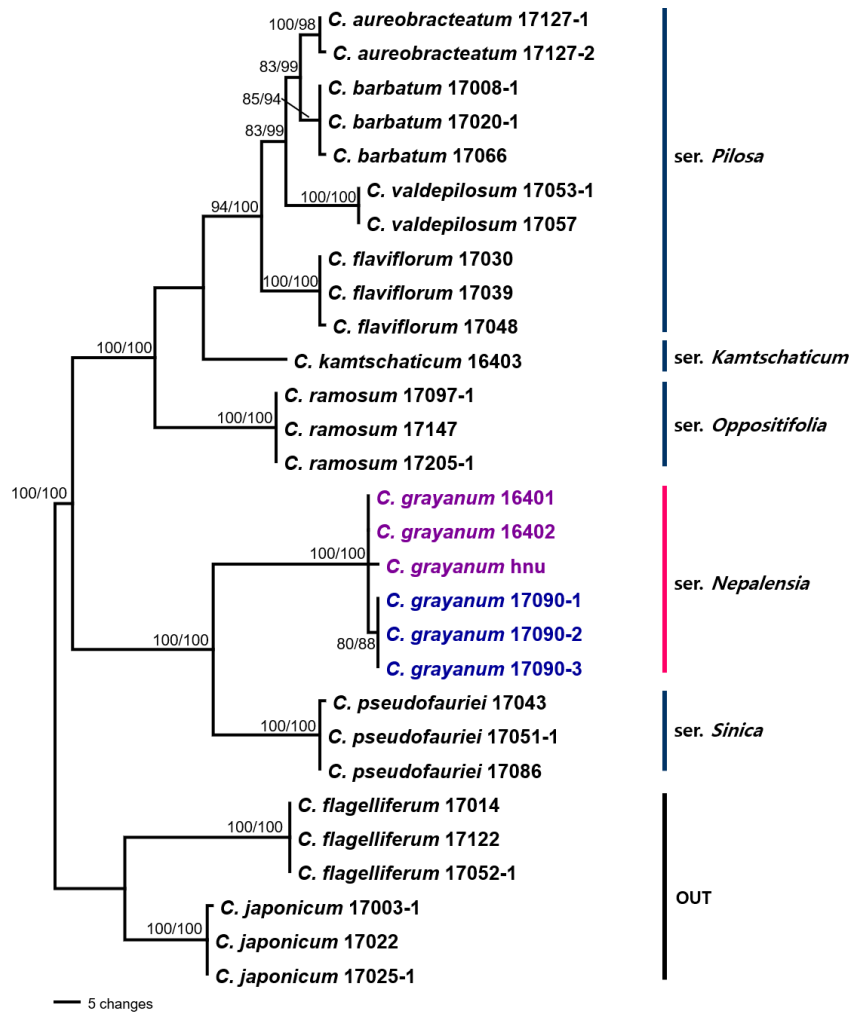


Fig. 4. Phylogenetic relationships resulting from the maximum parsimony analysis of nrITS sequences from eight *Chrysosplenium* taxa and two outgroup taxa (*C. flagelliferum* in ser. *Flagellifera* and *C. japonicum* in ser. *Alternifolia*). Numbers above the branches indicate bootstrap values (≥ 80) for maximum parsimony (left) and neighbor-joining (right) analysis.

Key to taxa of *Chrysosplenium* modified from Kim (2007)

- 1. Leaves alternate.
 - 2. Leaves heterophyllous; sterile branches developed; caluline and bracteal leaves 2–5 lobed *C. flagelliferum* (애기괭이눈)
 - 2. Leaves isophyllous; sterile branch absent; cauline and bracteal leaves not lobed with 8–12 teeth.
 - 3. Stolons present; bracteal leaves bright yellow at flowering; seed surface smooth *C. alternifolium* (시베리아괭이눈)
 - 3. Stolons absent; bracteal leaves greenish yellow at flowering; seed surface minutely papillose *C. japonicum* (산괭이눈)
- 1. Leaves opposite.
 - 4. Sepals green, spreading; capsules cup-shaped
 - *C. ramosum* (가지괭이눈)
 - 4. Sepals yellow, erect; capsules horn-shaped.
 - 5. Plants glabrous or pubescent at some parts.
 - 6. Plants glabrous; stamens 4(–6); cylindrical papillae with roundish head at the tip on smooth seed *C. grayanum* (괭이눈)
 - 6. Plants glabrous except petiole of rosette leaves; stamens 8; cylindrical papillae with truncate tip on scabrous seed surfaces *C. pseudofauriei* (선괭이눈)
 - 5. Plants pubescent at all parts.
 - 7. Seeds without tubercules.
 - 8. Leaves of sterile branches congested at distal end, with white variegated veins on upper surface *C. flaviflorum* (누른괭이눈)
 - 8. Leaves of sterile branches distantly arranged, with silvery dotted upper surface

-*C. epigealum* (기느괭이눈)
7. Seeds with tubercles.
9. Seed tubercles arranged on inconspicuous longitudinal ridges.
10. Sterile branches highly branched, ca. 30 cm long after fruiting; leaves of sterile branches with silvery dots, upper surface glabrous; bracteal leaves yellowish-green
.....*C. ramosissimum* (가지털괭이눈)
10. Sterile branches unbranched, less than 15 cm long after fruiting; leaves of sterile branches without silvery dots, upper surface pilose; bracteal leaves bright yellow
.....*C. valdepilosum* (금괭이눈)
9. Seed tubercles arranged on prominent longitudinal ridges.
11. Leaves of sterile branches distantly arranged after fruiting; bracteal leaves golden yellow, greenish yellow at flowering
.....*C. aureobracteatum* (연노랑괭이눈)
11. Leaves of sterile branches congested at distal end after fruiting; bracteal leaves green at flowering
.....*C. barbatum* (흰털괭이눈)

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