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Research Article

Sisymbrium irio L. (Brassicaceae): a new alien plant in Korea

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Abstract

As anthropogenic transports of biomaterials have rapidly escalated in the past century, biological invasions have been of great concern on a global scale. Given the exponential growth of alien species passing through the initial point of introduction, reporting an alien species on its initial appearance is important. *Sisymbrium irio*, an annual herb distributed widely throughout the northern hemisphere, is a notorious weed for its strong invasiveness. In 2016 *S. irio* was first reported in Korea. The plant was found along the urban expressway, Beonyeong-ro, connecting Busan trading port to Gyeongbu expressway. To properly identify the species, we collected ten *S. irio* samples from the reported site and examine their morphological characters. The identification key of *Sisymbrium irio* and its related species in the genus was constructed to clarify taxonomic confusions. We further determined the identity of the taxa by inferring the phylogeny of *S. irio* and the related taxa. To investigate the distribution changes for the past four years since the first observation in 2016, we monitored the area in late summer of 2020. Both morphological and molecular examinations indicated that the *Sisymbrium* species we found in Korea for the first time was *S. irio*. From our site monitoring, we newly found a second distribution site that is approximately 1.5 km away from the original site. Considering the geographical proximity of the introduction sites from the port, we hypothesize that *S. irio* might started expanding its range by the aid of roads. Given the role of roads as a corridor for alien species, further expansion of *S. irio* in Korea is expected.

Key words: invasive plant, unrecorded plant, alien species, range expansion, phylogeny

Introduction

Sisymbrium irio L. (Brassicaceae), a notorious weed for its strong invasiveness, is an annual herb distributed widely throughout the northern hemisphere. In Korea, six species including *S. irio* have been recognized in the genus *Sisymbrium* L. Notably, only two of the six taxa [*S. heteromallum* C.A.Mey., *S. luteum* (Maxim.) O.E. Schulz] are native in Korea, while the remaining four [*S. altissimum* L., *S. irio, S. officinale* (L.) Scop., *S. orientale* L.] are alien species (Chang et al. 2017). Due to the increased reproductive potential such as the large seed production and a series of competitive physiological

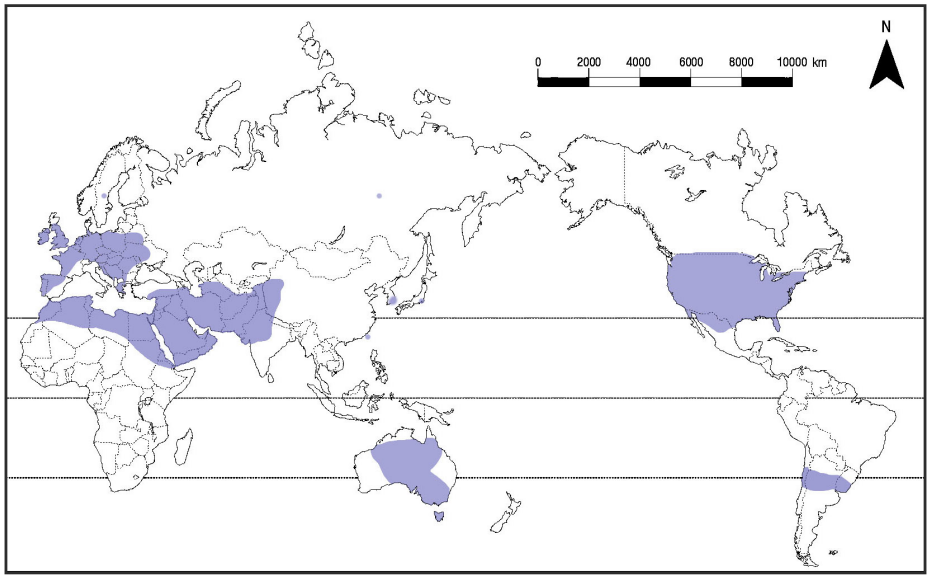

Figure 1. Global distribution of *Sisymbrium irio* on a world map. The lilac shadings indicate the occurrences of the species across the globe. Korea was newly added to the occurrence point based on the study. The occurrence point on the map was modified from the CABI website (https://www.cabi.org/isc/datasheet/50196).

characteristics, the family Brassicaceae is well known for its invasiveness (Chauhan et al. 2006; Dellow et al. 2006; Ray et al. 2005; Warwick et al. 2002). Likewise, *Sisymbrium* species exhibit strong invasiveness as shown in the increased percent number of invasive taxa in Korea, i.e. over half of the *Sisymbrium* species appear to be invasive in Korea.

Sisymbrium irio, so-called "London Rocket", is a yellow-flowered weed that is native to southern Europe and North Africa. The plant has widely been naturalized in North America, South America, Australia, South Africa, and Asian countries (China, India and Japan; CABI 2020; Ray et al. 2005; USDA 2020; See Figure 1 for the distribution). The use of *S. irio* has been common for medicinal purposes to treat coughs, rheumatism, inflammation and other diseases (Al-Jaber 2011; Bagheri et al. 2019; Hailu et al. 2019). The seeds have also been used for making beverages and oils (Al-Jaber 2011; Hailu et al. 2019). The plant is well famed for its dietary advantages due to its rich nutrients and the abundant protein contents (Guil-Guerrero et al. 1999). Despite the diverse usages, like other *Sisymbrium* species, *S. irio* is a notorious invasive plant with several biological characters promoting invasion success such as the increased number of flowers and seeds (Chauhan et al. 2006; Ray et al. 2005). Due to the high invasiveness, the species is regarded as an aggressive weed to be controlled in many countries including the United States, Australia and Russia (CABI 2020; Dellow et al. 2006; Tretyakova 2011).

In Korea, the existence of *Sisymbrium irio* was first confirmed during a field survey of the Sooyoung riverside (35°12′31.4″N; 129°07′13.4″E), Busan in 2016. In the current study, we report the new record of *S. irio* and its habitat characteristics in Korea. We carefully determine the identity of the species using both morphological and molecular examinations. We then

Table 1. Voucher information and GenBank accession numbers for 19 accessions of *Sisymbrium irio* and closely related taxa examined in this study (outgroup taxa included). All vouchers are deposited in KH except sequences downloaded from Genbank.

Accession	Locality	Genbank accession number	
		*rbc*L	*mat*K
Sisymbrium altissimum			
SA1	Korea. Cungcheongnam-do, Asan-si	MW174824	MW188523
SA2	Korea. Gteonggi-do, Pocheon-si	MW174825	MW188524
SA3	Korea. Gangwon-do, Pyeongchang-gun	MW174826	MW188525
SASSC J04*	Pakistan.	NC_037838	
Sisymbrium irio			
SI1	Korea. Busan-si, Haeundae-gu	MW174821	MW188520
SI2	Korea. Busan-si, Haeundae-gu	MW174822	MW188521
SI3	Korea. Busan-si, Haeundae-gu	MW174823	MW188522
Sisymbrium luteum			
SL1	Korea. Kyeongsangbuk-do, Yeongyang-gun	MW174830	MW188529
SL2	Korea. Gangwon-do, Pyeongchang-gun	MW174831	MW188530
SL3	Korea. Gangwon-do, Jeongseon-gun	–	MW188531
SL4	Korea. Gangwon-do, Jeongseon-gun	–	MW188532
Sisymbrium officinale			
SF1	Korea. Kyeogsangbuk-do, Ulleung-gun	MW174827	MW188526
SF2	Korea. Jeju-do, Jeju-si	MW174828	MW188527
SF3	Korea. Jeju-do, Jeju-si	MW174829	MW188528
Sisymbrioum orientale			
SO1	Korea. Jeollabuk-do, Gunsan-si	MW174832	MW188533
SO2	Korea. Jeju-do, Jeju-si	–	MW188534
SO3	Korea. Gyeongsangnam-do, Yangsan-si	–	MW188535
Brassica napus			
ZY036	China. Cultivated	NC_016734	
Arabis josiae			
sn.	Not specifed	NC_049580	

* Sequences downloaded from Genbank.

provided morphological descriptions and a key to the allied taxa, illustrations, and photographs to fully characterize the newly discovered invasive alien plant in Korea. The information derived from our study may offer directions for monitoring and management for the new putative invasive species in Korea.

Materials and methods

Sample collection

Sisymbrium irio and five morphologically related *Sisymbrium* species distributed in South Korea were collected from 13 populations (see Table 1 for the detailed sample information) for morphological and molecular analyses. In the molecular analysis, *S. heteromallum* was excluded because collecting the sample was challenging due to its distribution (throughout East Asia, exclusively located in the northern part of Korea). For the molecular analyses, we assigned two Brassiceae species (*Brassica napus, Arabis verna*) to the outgroup based on previous phylogenetic research (Warwick et al. 2002). We also downloaded a South Asian *S. irio* sample sequence from Genbank (see Table 1 for the accession number).

Morphological examination and expansion monitoring

We examined the morphological characters using the ten collected *Sisymbrium irio* samples and 30 herbarium specimens of 5 *Sisymbrium* species distributed in Korea that were deposited in Korea National Arboretum (KH). We also visited 5 virtual herbaria (HUH, WUK, NAS, PE, MNHN; see the herbarium specimens examined in the results) and searched for specimen photographs of the five taxa. We then compared the key characters such as basal leaves, petal apex, fruiting pedicels and inflorescence among the 5 taxa. For the morphological parts, an Olympus dissecting stereo microscope (SZX16) was used. To illustrate the key characters, we photographed the plant parts (flower, pistil, stigma, base of pistil, stamen, petals, sepals, fruit) and draw the detailed features (Figure 4). To investigate the distribution changes during the past four years since the first observation was made in 2016, we monitored the site in late summer of 2020. We surveyed the area by taking a linear walk along the roads and river sides for ~ 5 km. During the walking survey, we checked the presence of the species and recorded the GPS coordinates upon each observation. Each population found was characterized by its size and geographic characteristics.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from either fresh or dried leaf samples, using DNeasy plant mini kit (Qiagen, Germany) followed by the manufacturer's protocol. We selected two regions of cpDNA, *rbc*L and *mat*K after evaluating DNA polymorphism level on three cpDNA markers. *trn*L-*trn*F IGS was not selected for the full analyses because the test analysis exhibited nearly zero polymorphism among the 5 test samples. The PCR amplifications were carried out using GeneAmp PCR system 9700 with a total reaction volume of 20 uL containing 50ng template DNA. The amplification conditions are provided in Supplementary material Table S1. The amplified fragments were then sent to Macrogen Inc. (South Korea) for sequencing. The briefly summarized sequencing steps were as followings: after a series of purification steps, the PCR products were sequenced on an ABI Prism 3730XL genetic analyzer (Applied Biosystems, USA) using ABI Prism BigDye® terminator v 3.1 cycle sequencing kit (Applied Biosystems, USA).

Sequence alignment and analysis

All sequences were edited and aligned using Geneious Aligner in Geneious Prime ver. 2020.0.5, while other parameters were set as defaults. We then manually adjusted the aligned sequences. All DNA sequences obtained from this study were deposited in Genbank (See accession numbers in Table 1).

We conducted the phylogenetic analyses in two manners: a) based on the two cpDNA regions independently and b) based on the concatenated

cpDNA sequence of the two regions. The phylogenetic trees were inferred from two tree building algorithms, maximum parsimony (ML) and Neighbor-Joining (NJ). ML analyses were performed using RAxML plugin v4.0 implemented in Geneious Prime with the GTR CAT model (Stamatakis 2014). Node supports were evaluated with 1000 bootstrap replicates (Felsenstein 1985). We constructed the Neighbor Joining tree using PAUP plugin implemented in Geneious Prime with 1000 bootstrap replicates to estimate the robustness of each node. The nucleotide substitution model used for the tree reconstruction was the Kimura-2 parameter model (K2P) (Kimura 1980).

Results

Habitats and distribution changes

The first official record of *S. irio* was made in 2016 (35°12′31.4″N; 129°07′13.4″E). However, according to unofficial records made by a local amateur botanist, a few individuals were already observed in 2012. At the time of field survey in 2016, hundreds of *S. irio* were growing in ~ 1 × ~ 200 m rectangular shape habitat. We found the second locality of *S. irio* from the monitoring results of 2020. During the 2020 monitoring, we learned that the original habitat was destroyed by bridge construction, thus the original population has dramatically been reduced to about less than 30 individuals. The second locality that we found covered an area of ~ 20 m × 1 m, which was ~ 1.5 km away from the original habitat (35°12′34.7″N; 129°07′13.5″E; Figure 2). More than a hundred individuals were growing in the new site.

Descriptions of morphological characters for Sisymbrium irio *found in Korea*

***Sisymbrium irio* L.**

(Figures 3 and 4).

Type: Spain: *836.35* [Lectotype: LINN!, designated by Jonsell and Jarvis, Nordic J. Bot. 22: (2002)].

= *Sisymbrium pinnatifidum* Forsskål (1775: 118).
= *Sisymbrium irioides* Boissier (1842: 76).
= *Sisymbrium maximum* Hochstetter ex Fournier (1865: 72).
= *Arabis charbonnelii* Léveillé (1913: 100).

Herb annual or winter annual, 20–60 cm tall. **Stems** erect, branched below and above, glabrous or sparsely pubescent at base. **Leaves** at the base withered after flowering and during fruiting; cauline leaves alternate; petiole 1–4.5 cm long; blade oblanceolate or oblong, runcinate-pinnatifid, 3–12 cm long, 1–6 cm wide; terminal lobe largest; middle cauline leaves oblong or lanceolate, margin entire, dentate, or lobed, both surfaces glabrous; uppermost cauline leaves smaller, simple or 1–3-lobed **Inflorescences** a 10–25 flowered, corymbiform raceme, pubescent. **Flowers** in a usually simple

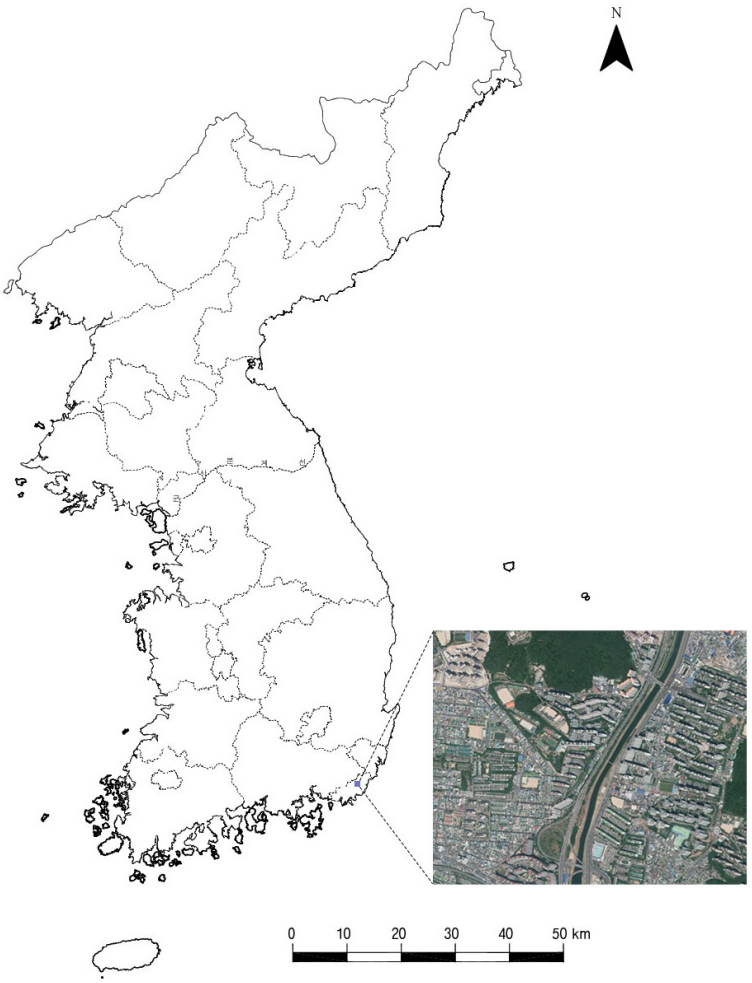

Figure 2. The locations of *Sisymbrium irio* L. in Korea. The plant was found on the slope of expressway alongside river.

raceme; sepals 4, oblong, 2–2.5 mm long, 1–1.5 mm wide; petals 4, bright yellow, oblong to oblanceolate, 2.5–4 mm long, 1–1.5 mm wide, slightly pubescent; claws 1–1.5mm long; filaments yellow, 2.5–4 mm long; anthers oblong, 0.5–0.9 mm long; style 2–2.5 mm long; stigma 2-lobed; ovules 40–90 per ovary. **Silique** slender, linear, terete, straight or slightly curved inward, 3–4 cm long, 0.9–1.1 mm wide; fruiting pedicels much slender than fruit, 0.5–2 cm long; young fruits overtopping inflorescence; **Seeds** yellowish-brown, oblong, 0.8–1 mm long, 0.5–0.6 mm wide.

Phenology: Flowering time March (February) to May and fruiting time March to June.

Vernacular (Korean) name: *Git-teol-jang-dae* (new Korean name).

Habitat and ecology: Abandoned fields, roadsides, pasture, livestock watering sites, ditches, riparian areas, orchards, fence rows, open desert landscapes and disturbed habitats (Shehata 2014). In Korea, based on field observations, this species was found on the sunny slope of an expressway. It grows with both the cultivated: *Brassica napus* L., *Euonymus japonicus* Thunb., *Lycium chinense* Mill., *Prunus* × *yedoensis* Matsum., *Trachelospermum asiaticum* (Siebold & Zucc.) Nakai and the wild: *Artemisia*

Figure 3. Photographs of parts of *Sisymbrium irio* L. A. Growth form, B. Root, C. Basal stems, D. Young plants (not rosulate), E. Flowering stage plant, F. Inflorescence, G. Flowers (over-view), H-I. Seeds (dried) (H: adaxial view, I: lateral view). The images were photographed by Yoon-Young Kim and Bong-Sik Lee.

indica Willd., *Bidens subalternans* DC., *Bromus catharticus* Vahl, *Bromus rigidus* Roth, *Cerastium glomeratum* Thuill., *Chenopodium album* var. *centrorubrum* Makino, *Commelina communis* L., *Corchoropsis tomentosa*

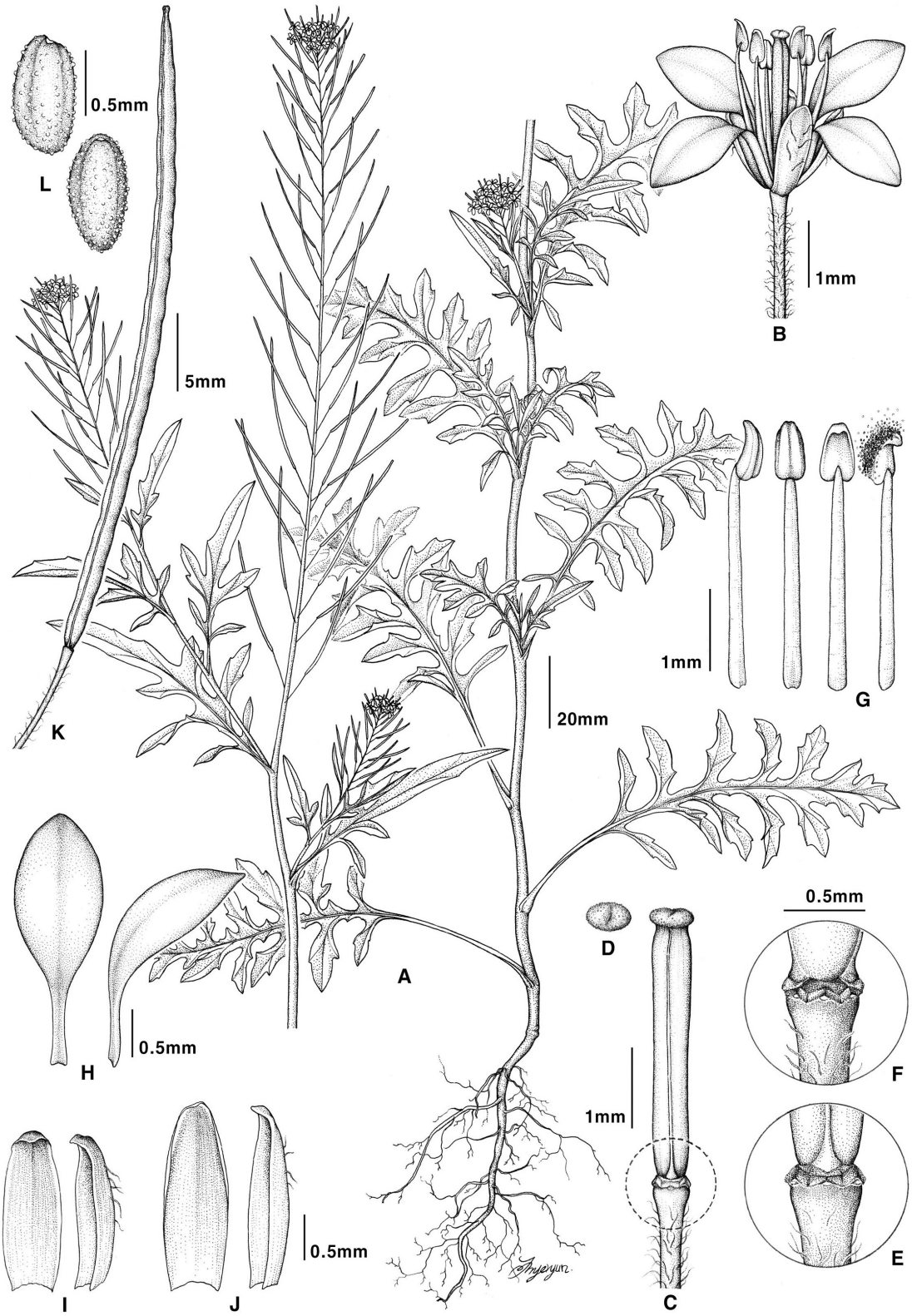

Figure 4. Illustrations of *Sisymbrium irio*. A. Habitat, B. Flower, C. Pistil, D. Stigma, E-F. Base of pistil (E: front view, F: lateral view), G. Stamen, H. Petals, I-J. Sepals (I: short type, J: long type), K. Fruit (Silique), L: Seeds. Illustrations by Hyeryun Jo.

(Thunb.) Makino var. *psilocarpa* (Harms & Loes. ex Gilg & Loes.) C.Y.Wu &Y.Tan, *Erigeron annuus* (L.) Pers., *Erigeron bonariensis* L., *Forsythia koreana* (Rehder) Nakai (cultivated), *Hemistepta lyrata* (Bunge) Bunge, *Juniperus chinensis* L., *Lamium amplexicaule* L., *Lolium perenne* L.,

Oenothera biennis L., *Oxalis stricta* L., *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch., *Rorippa palustris* (L.) Besser, *Sonchus oleraceus* L., *Sonchus asper* (L.) Hill, *Stellaria media* (L.) Vill., *Taraxacum officinale* F.H.Wigg., *Trifolium repens* L, *Vicia angustifolia* L. ex Reichard var. *segetilis* (Thuill.) W.D.J. Koch.

Specimens examined: KOREA. Busan: Haeundae-gu, Banyeo-dong, riverside, 4 May 2016, *CSC160504*(KH), Busan: Haeundae-gu, Banyeo-dong, riverside, 7 April 2020, *HWK370*, *HWK370, HWK371, HWK372, HWK373, HWK374* (KH). **UNITED STAES OF AMERICA.** Texas: Del Rio, 13 Feb 1937, V.L. Cory *01716027* (HUH); Texas: Marathon, 12 March 1933, V. L. Cory 01716049 (HUH); California: Patterson, 25 March 2015, R.R. Halse, *00395151* (HUH); Nevada: Nye Co., Amargosa Desert, 23 April 1985, Ann Pinzl *01716016* (HUH); Arizona; Larrea-mesquite area, 9 miles north of Ajo, Maricopa County, 20 March 1993, Reed C. & Kathryn W. Rollins *01693985* (HUH); Pennsylvania: 14 June 1879, C.F. Parker 01716019 (HUH). **CHINA.** Xinjiang: Uygur autonomous region, 8 Sep. 1978, Xizhi Xinjiang Team *0341280* (WUK); Xinjiang: Gongliu County, 22 Aug. 1978, Xizhi Xinjiang Team *0338806* (WUK). Gansu Province: Pingliang city, 28 May 1974, Z. B. Wang 00329924 (NAS); Shaanxi Province: Jingbia County, 29 Jul. 1956, Hangha team *02078347* (PE). **INDIA.** Rawalpindi: 15 Nov. 1932, T. N. Lion *01160139* (PE); Delhi: University gardens, 2 Jul. 1955, S.C. Chadha *01160141* (PE). **Iraq.** Mesopotamie: 1837, P.M.R. Aucher-Eloy *P-P02272615* (MNHN); s. loc., Láppi *P-P02272613* (MNHN). **FRANCE.** Paris: Museum entrance – angle r. Buffon and Bd of hospital, 17 Jul. 1958, P. Jovet *P-P00050210* (MNHN). **Mexico.** Ixmiquilpan: 16 March 1966 *P-P04629676* (MNHN); s. loc., E.G. Camus *P-P00318708* (MNHN). **TURKEY.** Prope Smyrnam: 1721, W. Sherard *P-P02272614* (MNHN).

Taxonomic notes: *Sisymbrium irio* is delimitated from the closest relative, *S. loeselii* L., by being glabrous or slightly pubescent throughout the plant and young fruits are overtopping flowers. Comparing to native Brassicaceae, before flowering, it is similar to *Rorippa sylvestris* (L.) Besser but different in having deeply lobed leaves and no rhizome. When the basal and cauline leaves withered after flowering, *S. irio* looks like *Descurainia sophia* (L.) Webb ex Prantl but it is distinguished by having no stellate hairs. The key of taxa in the genus *Sisymbrium* in Korea is presented below. Major morphological characters are the presence of basal leaves, branching, angle of fruiting pedicels, length of young fruit and surface of the silique.

Key of *Sisymbrium* in Korea

1. Fruiting pedicels as thick as fruit 2
– Fruiting pedicels slender than fruit 3
2. Stems glabrous; silique spreading; uppermost cauline leaves not divided into linear or filiform segments ..... *Sisymbrium orientale*

– Stems sparsely hirsute or not; silique ascending; uppermost cauline leaves pinnately divided into linear or filiform segments ..... *Sisymbrium altissimum*

3. Cauline leaves dentate; silique smooth or torulose 4
– Cauline leaves pinnatisect; silique slightly torulose 5
4. Basal leaves absent; only lower cauline leaves pinnately lobed, cauline leaves elliptic; raceme with basal bract, silique smooth ..... *Sisymbrium luteum*
– Basal leaves rosulate; cauline leaves oblanceolate; raceme without basal bract; silique torulose ..... *Sisymbrium heteromallum*
5. Basal leaves rosulate; petal apex rounded; fruiting pedicels appressed to rachis, slightly winding; young fruit not overtopping inflorescence ..... *Sisymbrium officinale*
– Basal leaves absent; petal apex slightly acuminate; fruiting pedicels ascending; young fruit overtopping inflorescence ..... *Sisymbrium irio*

Molecular phylogenetic analysis

A total of 28 sequences of the two cpDNA regions were obtained from 16 accessions of *S. irio* and related taxa. Sequence lengths of the two CP genes (*rbc*L and *mat*K) were 516 bp and 683 bp respectively (Table 2). The combined cpDNA set was 1199 bp in length after alignment (*rbc*L: 516 bp; *mat*K: 683 bp), of which 20 were variable and 16 were parsimoniously informative (Table 2). GC ratio was 44.1% and 30.3% for *rbc*L and *mat*K respectively (Table 2). The polymorphism observed from the two CP genes was higher in *mat*K than the one found in *rbc*L (Table 2). K2p distance among ingroup individuals were 0–0.0098 (mean 0.0036) for *rbc*L and 0–0.0133 (mean 0.0072) for matK (Table 2).

The inferred NJ and ML trees from the two cpDNA regions were nearly identical in the overall topology (Figure 5). One exception was that the sequences of *rbcL* region for *S. altissimum* and *S. officinale* were identical resulting in a polytomy in *rbc*L phylogeny (Figures S1, S2). *Sisymbrium irio* individuals collected from Korea were always nested together with *S. irio* from Europe (Figures S1–S4). The NJ and ML trees from concatenated cpDNA data set were identical with *matK* tree (Figures 5, S3, S4).

Table 2. Statistics for the cpDNA data sets used in this study. Outgroup taxa were excluded in the calculation of these statistics.

	*rbc*L	*mat*K	Concatenated
Sequence length (bp)	516	683	1199
Aligned length (bp)	516	683	1199
mean G+C ratio (%)	44.1	30.3	36.2
No. of variable characters	6	14	20
No. of parsimony informative characters	5	11	16
K2P distance (mean)	0–0.0098 (0.0036)	0–0.0133 (0.0072)	–

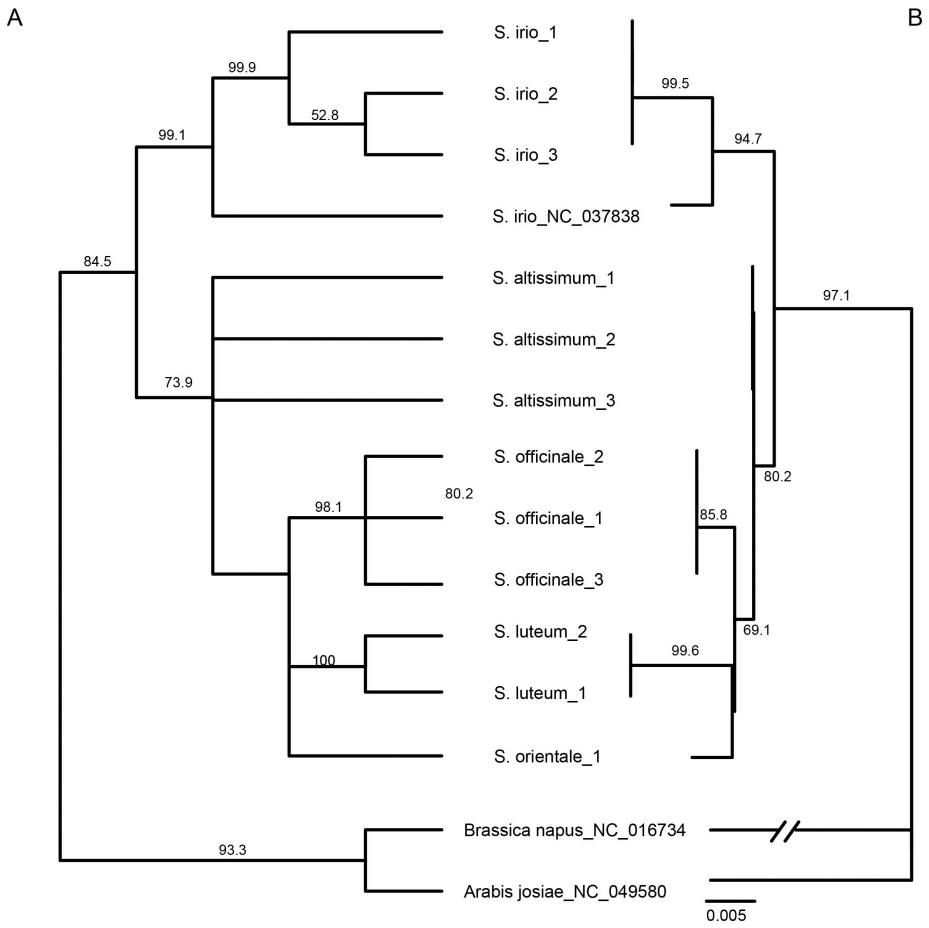

Figure 5. A. Maximum likelihood tree for *S. irio* and related taxa based on cpDNA *rbc*L and *mat*K regions. B. Neighbor Joining tree for individuals of *S. irio* and related taxa based on cpDNA *rbc*L and *mat*K regions. Numbers above branches are bootstrap values.

Discussion

Species invasions have grown rapidly as intercontinental travels have become frequent in the past century (Pimentel et al. 2000; Richardson et al. 2000). Although not all introduced species are successfully naturalized in their new environments and become problematic, even a small portion of the introduced species with high invasiveness can cause tremendous damages once they establish sustainable populations (Pimentel et al. 2000). Because the cost amending the damages is likely growing exponentially after the initial stages of invasion, reporting an invasive species early upon its first occurrence in the nature is critical to prevent it from further spreading. Our study intended to officially report the introduction of *Sisymbrium irio*, a notorious invasive plant, in Korea and investigate the potential range expansion of the species. Given the taxonomic difficulties (Khodashenas and Assadi 2007), the identification of the species requires a thorough diagnosis from both morphological and molecular approaches. Our study provided the morphological and molecular evidence of *S. irio*'s existence in Korea. The field monitoring results suggested the high potential of range expansion of the plant.

Sisymbrium irio was found on the slope of an urban expressway named Beonyeong-ro, which is the first urban expressway in Busan alongside Sooyoung river. It penetrates the city connecting Busan trading port to Gyeongbu Expressway and plays an important role for transmission of freight containers. The role of roads as a corridor for the movement of new species is quite well recognized (Hulme 2009; Kawamata et al. 2018). We hypothesized that the seeds of *S. irio* were unloaded at the port and accidentally released to the roadside. The fact that different alien species, i.e., *Bidens subalternans* (DC.) var. *subalternans* (Kim et al. 2012) and *Barbarea verna* (Mill.) Asch. (Hong et al. 2012) were also found near the same urban expressway may support our hypothesis. International trading ports are thought to be the major area of introduction for new alien species, thus a large number of alien species are often found naturalized in port areas (Hulme et al. 2008, Hulme 2009; Kawamata et al. 2018; Kim et al. 2019; Park 2009). *S. irio* propagules were likely transported by commercial ships and released near the international trading port. Subsequently, the highway might act as a conduit for the propagules.

Species invasions occur in a series of stages (Pyšek et al. 2004; Theoharides and Dukes 2007; Blackburn et al. 2011) and various factors influence their establishment and determine the success of invasion, including the following: biological characteristics, propagule pressure and residence time (Baker 1965; Williamson and Fitter 1996; Colautti et al. 2006; Pyšek et al. 2015). *Sisymbrium irio* is a winter annual plant and matures earlier than most annual plant species, which might offer high competitiveness over native plants during intrusion periods (Academy Village 2015; Chauhan et al. 2006; DiTomaso et al. 2013; Ray et al. 2005). The plant produces thousands of seeds per individual and the ground-buried seeds can survive up to 10 years (DiTomaso et al. 2013). Based on the classification of Richardson et al. (2000), *S. irio* is likely a naturalized plant rather than a potentially invasive or threatening species in Korea.

Our study is the first study that officially reports the introduction, naturalization and potential range expansion of *Sisymbrium irio* in Korea. The new occurrence found in our study is likely the evidence of successful naturalization of the species. It may also be the initiation point of range expansion; thus, expansion rate and the pattern of spread should be annually monitored to prevent the species from spreading over a wide range in Korea.

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Supplementary material

The following supplementary material is available for this article:

Table S1. PCR/sequencing primers and PCR cycling conditions for cpDNA regions examined in this study.
Figure S1. Neighbor Joining tree based on cpDNA *rbc*L regions.
Figure S2. Maximum likelihood tree based on cpDNA *rbc*L regions.
Figure S3. Neighbor Joining tree based on cpDNA *mat*K regions.
Figure S4. Maximum likelihood tree based on cpDNA *mat*K regions.

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