Actual distribution and present status of a threatened aquatic plant, *Sagittaria aginashi* (Alismataceae), in Korea

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ABSTRACT: We evaluated the actual distribution, present status, and number of remnant individuals of Korean *Sagittaria aginashi* (Alismataceae) based on herbarium specimens and field surveys. We also reidentified Korean *S. aginashi* by analyzing sequences of the internal transcribed spacer (ITS) region. We found nine specimens of *S. aginashi* in herbaria from four localities housed at the Osaka Museum of Natural History (OSA) and at the Korea National Herbarium (KH). During our field research, we could not confirm the current statuses of two collection localities (Taereung and Ansan-si), though this was not the case for Ulsan-si. In addition, we found two new localities in Ulsan-si and Yangsan-si. These three remnant populations are distributed in wetlands in mountainous areas (elev. 480–740 m). With regard to the number of flowering and immature individuals in the three localities, there were <50 and <2,000, respectively, in total. ITS sequences demonstrated that the sequences of Korean and Japanese *S. aginashi* are uniform and distinct from other *Sagittaria* species. These results indicate that *S. aginashi* is a rare and threatened species in Korea. It should be listed as an endangered species on the Korean Red List and requires urgent protection by conservation programs, including the extensive surveys of other possible natural habitats.

Keywords: Genetic identification, ITS, Korea, Red List, *Sagittaria aginashi*

Aquatic macrophytes are an important ecological element in inland water ecosystems. However, because their general habitats are highly sensitive to environmental change caused by anthropogenic impact (such as eutrophication, water abstraction, sediment mining, shoreline reinforcement, and invasive alien species), massive diversity loss has occurred (Cronk and Fennessy, 2001; Akasaka et al., 2010; Kadono, 2014). To develop an optimum conservation program, it is necessary to correctly identify species and recognize their present status (Frankham et al., 2010). Taxonomic status must be accurately established to ensure that neither are endangered species denied protection nor is effort wasted on abundant species. Many aquatic macrophytes showing highly adaptive, phenotypic plasticity, and morphology among juvenile and adult plants often have highly different characteristics (Sculthorpe, 1967). It is occasionally difficult to identify aquatic plant species lacking taxonomic key characters based on only morphological features.

For aquatic macrophytes, taxonomic misidentification may lead to ignoring populations of threatened species requiring conservation programs. In addition, incorrect conservation effort may seriously affect natural populations.

The genus *Sagittaria* L. (Alismataceae) is a group of perennial, submerged to emergent aquatic plants, consisting of approximately 36 currently recognized species (Keener, 2005).

The distribution area of the genus includes Africa, Asia, Europe, and North and South America. In Korea, three native *Sagittaria* species have been identified (Kim and Choi, 2007; Lee, 2007): (1) *S. aginashi* Makino; (2) *S. pygmaea* Miq.; and (3) *S. trifolia* L. In Korea, *S. trifolia* is sometimes treated as a synonym of *S. sagittifolia* L. (Kim and Choi, 2007). However, *S. sagittifolia* is different from *S. trifolia* in having petals with a violet spot at the base and violet anthers (Keener, 2005). No such species occurs in Korea, and therefore, we do not treat Korean *S. trifolia* as a synonym of *S. sagittifolia*.

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Sagittaria aginashi is a perennial emergent aquatic species, which is distributed in ponds, marshes, moors, and paddy fields in Korea and Japan (Kim and Choi, 2007; Lee, 2007; Kadono, 2014). In morphological traits of the emergent leaf and reproductive organs, *S. aginashi* is similar to *S. trifolia*, although *S. aginashi* is remarkable for the peculiarity that it has numerous small tubercles at the base within the vagina of petioles and no stolon (Makino, 1901; Kim and Choi, 2007; Kadono, 2014). For that reason specimens of *S. aginashi* lacking roots and the base of petioles have been often misidentified as *S. trifolia* (Shiga, unpubl. data). In these cases, DNA sequencing analysis is a powerful tool for identifying *Sagittaria* plants (Shiga et al., 2017).

In Japan, wetland destruction by human activity has led to the extinction of local populations of *S. aginashi*, and this species is categorized as a “near-threatened species” on the Japanese Red List (Ministry of the Environment Government of Japan, 2015). In Korea, *S. aginashi* is believed to be relatively common in almost all provinces (Shin et al., 1997) or in the eastern provinces (Gangwon-do, Gyeongsangbuk-do, and Gyeongsangnam-do) of the Korean peninsula (Kim and Choi, 2007). However, Choi (2000) has mapped this species in only one site of Gangwon-do. Recently, Oh et al. (2016) have published distributional maps of Korean plants based on specimen data, including *S. aginashi*, and the map revealed that the species may be extremely rare. Hence, detailed research into the actual distribution and present status of *S. aginashi* based on many specimens, including Japanese herbarium material and natural habitats, is needed to conserve this species.

The goal of the present study was to (1) estimate the actual distribution of *S. aginashi*, (2) evaluate the present status and number of individuals remaining in natural habitats of the species, and (3) re-identify Korean *S. aginashi* by analyzing sequences of the internal transcribed spacer (ITS) region. We also preliminary discuss the genetic relationships among related species.

Materials and Methods

Herbarium and field research

We surveyed herbarium specimens of *S. aginashi* collected in Korea and Japan deposited at the Korea National Herbarium (KH), the Kumamoto Prefecture Museum Network Center, Kobe University, Kyoto University (KYO), the National Museum of Nature and Science (TNS), Niigata University, the Osaka Museum of Natural History (OSA), and the University of Tokyo (TI). In KH, we investigated all specimens of the genus *Sagittaria*.

In September 2016, we also investigated the current population size and habitat conditions of *S. aginashi* in three remnant populations (Table 1). We counted the number of flowering and immature individuals.

ITS region analysis

To genetically identify the Korean *S. aginashi*, we conducted genetic analyses using the DNA sequence of the ITS regions (including ITS1, ITS2, and 5.8S rRNA regions) of nuclear ribosomal DNA. One plant was collected from each of the three remnant populations of *S. aginashi* and two herbarium specimens collected from Ansan-si (KH1269331) and Jeju Island (KH1058867) were surveyed (Table 1). The following six GenBank sequences were added to the DNA sequence dataset: *S. aginashi* (LC169553) collected in Japan, *S. lichuanensis* (AY395992), *S. potamogetifolia* (AY395990), *S. pygmaea* (LC169556), *S. tengtsungensis* (AY395994), and *S. trifolia* (LC169551) (Table 1). Sequence similarities among sequence types were calculated based on the ratio of the number of matched sequences among the number of sequences to be compared.

Total DNA was extracted from 50–100 mg of dried leaves of each specimen using the cetyltrimethylammonium bromide method (Milligan, 1992). Relevant portions of the ITS region were amplified from total genomic DNA by the polymerase chain reaction (PCR) using thermostable DNA polymerase (EmeraldAmp PCR Master Mix, Takara Bio Inc., Tokyo, Japan) and primers designed by White et al. (1990), namely ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR was performed using a TaKaRa PCR Thermal Cycler TP650 Standard (Takara Bio Inc.) programmed for 4 min at 94°C, 35 cycles of 1 min at 94°C (denaturation), 1 min at 53°C (annealing), and 1 min 30 s at 72°C (extension), with a final step of 7 min at 72°C. Direct sequencing of purified PCR products was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers on the ABI Prism 3130 automated sequencer (Applied Biosystems).

Results

Herbarium and field research

In our herbarium search, we found nine specimens of *S. aginashi* from four localities housed in OSA and KH (Fig. 1): Taereung (OSA), Ansan-si (KH), Ulsan-si (KH), and Jeju Island (KH). In KH, there were seven specimens of *S. aginashi* and many narrow-leaved *S. trifolia* had been misidentified as *S. aginashi*.

In our field research, we surveyed natural populations of *S. aginashi* based on the specimens' locality data. We could not confirm the current status of two collection localities (Taereung and Ansan-si), but only that of Ulsan-si (Mujechi-neup). We also found two new localities of *S. aginashi* in Ulsan-si (Doltimi-neup) and Yangsan-si (Mt. Sinbul-san). These three remnant populations are found in wetlands in mountainous areas (alt. 480–740 m) (Fig. 2, Table 1), and details are as follows. In case of locality in Jeju Island, we have not accessed in this study.

Firstly, in Mujechi-neup, the numbers of flowering mature and immature plants of *S. aginashi* were <10 and 100–1,000, respectively (Table 1). The distribution area was ca. 160 m² and the total wetland area was 13,000 m². *Sagittaria aginashi* plants are sympatric with *Utricularia bifida* L., *U. caerulea* L., *Isachne globosa* (Thunb.) Kuntze, *Miscanthus sinensis* Andersson, and juvenile *Alnus japonica* (Thunb.) Steud. Secondly, in Doltimi-neup, the numbers of flowering mature and immature plants were 20–30 and 50–100, respectively (Table 1). *Sagittaria aginashi* was distributed in an area of ca. 130 m² and the total wetland area was 400 m². The species are sympatric with *Scirpus wichurae* Boeck., *Schoenoplectiella*

hotarui (Ohwi) J. D. Jung et H. K. Choi, and *Saussurea pulchella* (Fisch. ex Hornem.) Fisch. Finally, in Mt. Sinbul-san, the numbers of flowering mature and immature plants were <10 and 100–1,000, respectively (Table 1). The species that are sympatric with *S. aginashi* were *M. sinensis* and *S. wichurae*. The distribution area of *S. aginashi* and the total wetland area were ca. 290 m² and 11,000 m², respectively.

Genetic identification and genetic variation

Among the DNA sequences of the ITS region (670–677 bp) from 11 samples of genus *Sagittaria*, six sequence types (A–F) were detected, distinguishable by 42 single nucleotide polymorphisms and five insertions/deletions (Table 2). The sequences of all *S. aginashi* from the three extant populations and the two herbarium specimens collected from Korea (Ansan-si and Jeju Island), as well as from GenBank data based on Japanese samples, were identical (sequence type A) (Table 1). Furthermore, the other sequence types each corresponded to a different species (sequence type B: *S. tengtsungensis*, C: *S. lichuanensis*, D: *S. pygmaea*, E: *S. potamogetifolia*, F: *S. trifolia*). The sequence-similarities between *S. aginashi* (sequence type A) and the other five species were 99.56%, 99.41%, 98.97%, 96.76%, and 95.58% for *S. tengtsungensis*, *S. lichuanensis*, *S. pygmaea*, *S. potamogetifolia*, and *S. trifolia*, respectively (Table 2).

Discussion

Sagittaria aginashi is remarkably peculiar because of the following characteristics: obtuse apex of basal lobes of sagittate leaves (Fig. 2F), small numerous tubercles at the base within the vagina of petioles (Fig. 2G), and no stolon (Fig. 2I) (Makino, 1901; Kadono, 2014). However, almost all specimens identified as *S. aginashi* did not have these key characters (T. Shiga, pers. obs.). Based on the Korean literature (e.g., Shin et al., 1997; Choi, 2000; Lee, 2003; Kim and Choi, 2007; Lee, 2007), *S. aginashi* probably have not been identified clearly in Korea. In fact, the photographs of *S. aginashi* in Lee (2003) and Lee (2007) are actually those of *S. trifolia*.

Sagittaria aginashi has sometimes been treated taxonomically as the same as *S. trifolia* (Keener, 2005). The ITS sequences distinguish the species of the genus *Sagittaria* and demonstrate that the sequences of *S. aginashi* are clearly different from those of *S. trifolia* (Table 1). We conclude that *S. aginashi* is rare, but clearly is distributed in Korea. We confirm that the ITS sequences of *S. aginashi* are uniform in the current preliminary genetic study (Tables 1, 2). To clarify the historical biogeography of this species, further genetic

Fig. 1. Distribution of *Sagittaria aginashi* Makino in Korea.

Fig. 2. *Sagittaria aginashi* Makino from Korea. **A.** Staminate flower. **B.** Small, closed pistillate flower. **C.** **D.** Fruits. **E.** Morphological variation of the leaf. **F.** Apex of basal lobes of sagittate leaves. **G.** Small tubercles in axil. **H.** *S. aginashi* in its habitat. **I.** The specimen from H (Tsubota et al. 44).

Table 1. A list of sampling localities and present status of *Sagittaria aginashi*. Accession numbers of internal transcribed spacer (ITS) sequences are also shown.

Taxon	Locality	Latitude	Longitude	Elevation (m)	No. of flowering plants	No. of immature plants	Distribution range (m ²)	Wetland size (m ²)	Accession no.	Sequence type	Voucher
<i>S. aginashi</i>	KOREA: Mujechi-neup, Ulju-gun, Ulsan-si	35°27'N	129°08'E	530	1–10	100–1,000	160	13,000	LC278398	A	<i>Tsubota et al. 44</i> (NGU4126)
<i>S. aginashi</i>	KOREA: Doltimi-neup, Buk-gu, Ulsan-si	35°40'N	129°22'E	480	20–30	50–100	130	400	LC278399	A	<i>Tsubota et al. 50</i> (NGU4127)
<i>S. aginashi</i>	KOREA: Mt. Sinbul-san, Yangsan-si, Gyeongsangnam Prov.	35°25'N	129°00'E	740	1–10	100–1,000	290	11,000	LC278400	A	<i>Tsubota et al. 54</i> (NGU4128)
<i>S. aginashi</i>	KOREA: Sangnok-gu, Ansan-si, Gyeonggi Prov.	37°17'N	126°55'E	80	-	-	-	-	LC278401	A	<i>Jeon 91244</i> (KH1269331)
<i>S. aginashi</i>	KOREA: Namwon-eup, Seogwipo-si, Jeju Prov.	33°21'N	126°41'E		-	-	-	-	LC278402	A	<i>Kang s.n.</i> (KH1058867)
<i>S. aginashi</i>	JAPAN: Joetsu-shi, Niigata Pref.	37°09'N	138°21'E	20	-	-	-	-	LC169553	A	<i>Shiga 7518</i> (NGU298)
<i>S. tengtsungensis</i>	CHINA				-	-	-	-	AY395994	B	
<i>S. lichuanensis</i>	CHINA				-	-	-	-	AY395992	C	
<i>S. pygmaea</i>	JAPAN: Takatsuki-shi, Osaka Pref.	34°53'N	135°35'E	40–50	-	-	-	-	LC169556	D	<i>Shiga 9070</i> (NGU3012)
<i>S. potamogetifolia</i>	CHINA				-	-	-	-	AY395990	E	
<i>S. trifolia</i>	JAPAN: Awashimaura-mura, Niigata Pref.	38°27'N	139°14'E	50?60	-	-	-	-	LC169551	F	<i>Watanabe 69</i> (NGU308)

research is required based on samples from the entire distributional range using multiple polymorphic markers.

In Korea, *S. aginashi* often has not been distinguished taxonomically with narrow-leaved *S. trifolia* and is believed to be a relatively common species. Although we have not surveyed on habitats of Jeju Island, we show that the number of populations of *S. aginashi* is quite low and that population sizes are extremely small in Korea (Fig. 1, Table 1). The lowland populations of the species may have become extinct mainly by human disturbance. The total number of flowering individuals in the three localities is <50, whereas that of immature individuals is <2,000 (Table 1). The effective population sizes of Korean *S. aginashi* may be quite small. The present status of *S. aginashi* indicates that this species should be listed as an endangered species in the Korean Red List and requires urgent protection by conservation programs including the extensive surveys on its additional natural habitats. Because *S. aginashi* reproduces asexually by numerous tubercles, intra-population genetic diversity may be

at quite a low level. An evaluation of the genetic diversity using polymorphic markers, such as microsatellite markers, is needed to formulate an appropriate conservation program.

Furthermore, *S. aginashi* (sequence type A) is closely related to the Chinese endemic species *S. tengtsungensis* (sequence type B) and *S. lichuanensis* (sequence type C) (Table 2). The genetic diversity and genetic relationships among Chinese endemic *Sagittaria* species including *S. tengtsungensis*, *S. lichuanensis*, and *S. potamogetifolia* have been investigated well using multiple genetic markers (Du et al., 1998; Keener, 2005; Chen et al., 2012; Liao et al., 2016). Liao et al. (2016) revealed that *S. tengtsungensis*, *S. lichuanensis*, *S. potamogetifolia*, and *S. pygmaea* form a monophyletic group and that *S. lichuanensis*, consisting of many sequence types, is a genetically polymorphic species. These species, except for *S. pygmaea*, are distinguishable from other *Sagittaria* species by forming numerous tubercles at the base of the petiole (Chen et al., 1984; Chen, 1989; Wang and Chen, 1996; Wang et al., 2010). To clarify the phylogenetic relationships and taxonomic

Table 2. Six sequence types distinguished by single nucleotide polymorphisms and insertion/deletions of internal transcribed spacers (ITS 1 and 2) and 5.8S rRNA regions in 11 samples of genus *Sagittaria* collected in Korea, Japan, and China. The numbers in parentheses after the type name indicate the number of samples analyzed.

Sequence type	ITS1																				5.8S						
																					3						
	1	2	5	6	6	6	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	3
1	3	0	1	2	3	7	1	2	5	0	8	4	3	3	9	2	3	0	0	5	6	7	8				9
A(6)	C	T	C	T	C	T	G	C	C	A	T	C	-	T	T	C	G	C	T	A	-	-	-	-			A
B(1)	C	T	C	T	C	G	G	C	C	A	T	C	-	T	T	C	G	C	T	A	-	-	-	-			A
C(1)	C	T	C	T	C	G	G	C	C	A	T	T	-	T	T	C	G	C	T	A	-	-	-	-			A
D(1)	C	T	C	T	C	G	G	C	C	G	A	C	-	T	A	C	G	C	C	A	-	-	-	-			A
E(1)	T	T	C	T	T	G	G	A	T	A	T	C	A	T	A	C	G	C	T	A	T	A	T	A			A
F(1)	C	C	A	C	C	G	C	C	C	G	T	C	-	C	A	T	A	T	T	G	-	-	-	-			T

Table 2. Continued.

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status of *S. aginashi* among these Chinese endemic species, phylogenetic studies based on the DNA sequences of multiple loci and detailed morphological studies are needed.

Taxonomic treatment

Sagittaria aginashi Makino, Bot. Mag. (Tokyo) 15: 104, 1901; *S. sagittifolia* L. var. *aginashi* Makino, Bot. Mag. (Tokyo) 6: 49, 1892. —TYPE: Japan, Honshu, Prov. Iwaki (Fukushima Prefecture), Maniwa near Sakamoto in Watari-ori, 17 Aug 1890, *Makino s.n.* [Lectotype designated by Tanaka (2005): MAK77766!] (Fig. 2).

Description: Perennial, glabrous herbs, 30–70 cm tall (29–150 cm —morphological variation ranges including Japanese plants indicated in parentheses); rhizomes erect, thick, densely rooting; stolon absent; corm absent; numerous tubercles on the inside of the sheath at base; tubercles 0.4–0.8 (0.3–1.0) cm long, 0.2–0.4 (0.2–0.7) cm wide. Leaves radical, fasciculate, erect, strongly nervate, petiolate; petioles 23–46 (23–83) cm long; blades lanceolate to linear or sagittate; leaves from spring to early summer and in juvenile plants lanceolate to linear without basal (lateral) lobes; basal lobes of sagittate leaves 7–12 (5–25) cm long; remainder of blade (middle lobe) 6–12 (4–20) cm long, 0.6–4.5 (0.6–9.8) cm wide; apex of basal lobes obtuse; latex seeping from scratches. Scapes erect, emerged, 30–71 (29–152) cm, taller than the leaves; inflorescence usually paniculate, in 4 to many whorls of (2 or) 3 flowers, lowest whorl rarely branched; female flowers on lowest 1 (or 2) whorls; bracteoles free or connate toward the base, 0.5–1.0 cm long. Flowers unisexual, the female flower below and male one above. Sepals 3, ovate to oblong, obtuse, entire, light green shaded with red, persistent, ca. 5–8 mm long. Petals 3, suborbicular, 8–13 mm long. Stamens 23 (18–24); anthers yellow. Ovaries numerous, apocarpous, crowded in a spherical head. Achenes 15–17 mm long, narrowly winged, with a short beak. Flower and fruit Jul–Aug. $2n = 22$ (Harada, 1956).

Korean name: Bo-pul.

Distribution and habitat: Japan (Hokkaido, Tohoku, Kanto, Chubu, Kinki, Chugoku, Shikoku, Kyushu) and Korea (Gyeonggi, Gyeongnam, Jeju) (Fig. 1). In ponds, lakes, marshes, moors, and paddy fields.

Specimens examined: KOREA. Gyeonggi: Taereung, 8 Sep 1940, *H-D. Jang* 1135, 1142 (OSA227615, 227616); Ansan-si, Sangnok-gu, 17 Sep 2009, *E. S. Jeon* 91244 (KH1269331). Ulsan-si: Ulju-gun, Somdong-myeon, Mujechi-neup, 17 Aug 2006, *S. H. Park & G. H. Nam* 61296 (KH1120514–1120516); 1 Sep 2016, *K. Tsubota et al.* 44, 45 (Niigata University = NGU4126, 4491); Buk-gu, Maegok-dong, Doltimi-neup, 1 Sep

2016, *K. Tsubota et al.* 46–52 (NGU4127, 4492–4497). Gyeongnam: Yangsan-si, Wondong-myeon, Dae-ri, 2 Sep 2016, *K. Tsubota et al.* 53–57 (NGU4128, 4498–4501). Jeju: Seogwipo-si, Namwon-eup, 23 Aug 2001, *Y. J. Kang s.n.* (KH1058229, 1058866, 1058867).

JAPAN. Hokkaido Pref.: Tomakomai-shi, 14 Aug 2016, *K. Tsubota et al.* 34–38 (NGU4124, 4484–4487). Aomori Pref.: Higashidori-mura, 5 Aug 2016, *S. Nemoto* 3369 (NGU4123); Goshogawara-shi, 14 Sep 2016, *K. Tsubota & A. Matsumoto* 70–72 (NGU4132, 4512, 4513). Akita Pref.: Mitane-cho, 15 Sep 2016, *K. Tsubota* 73–77 (NGU4133, 4514–4517). Iwate Pref.: Takizawa-shi, 13 Sep 2016, *K. Tsubota & T. Shiga* 65–69, 83 (NGU4131, 4507–4511); Isawa-cho, 1 Sep 1985, *Y. Kadono* 770 (Kobe University). Miyagi Pref.: Osaki-shi, 12 Sep 2016, *K. Tsubota & T. Shiga* 62–64 (NGU4130, 4505, 4506). Fukushima Pref.: Kitashiobara-mura, 27 Sep 2016, *K. Tsubota & K. Shutoh* 81, 82 (NGU4135, 4520); Tamakawa-mura, 1 Aug 2016, *K. Tsubota et al.* 27–29 (NGU4121, 4479, 4480); Iwaki-shi, 1 Aug 2016, *K. Tsubota et al.* 24–26 (NGU4122, 4477, 4478). Tochigi Pref.: Nasukarasuyama-shi, 29 Jul 2016, *K. Tsubota et al.* 17–22 (NGU4118–4120, 4474–4476); Hongo-mura, 15 Aug 1953, *K. Ogawa s.n.* (OSA). Niigata Pref.: Sado-shi, 16 Jul 2016, *K. Tsubota* 5–9 (NGU4117, 4470–4473); Gosen-shi, 25 May 2016, *K. Tsubota* 1–3 (NGU4466–4468), 7 Sep 2016, *K. Tsubota* 58–61 (NGU4129, 4502, 4504); Sanjo-shi, 25 May 2016, *K. Tsubota* 4 (NGU4469); Joetsu-shi, 25 Sep 2016, *K. Tsubota et al.* 78–80 (NGU4134, 4518, 4519), 9 Sep 2013, *T. Shiga* 7518 (NGU297, 298, 300), 24 Sep 2008, *T. Shiga & S. Takebayashi* 6388 (OSA239165). Nagano Pref.: Hakuba-mura, 19 Aug 1975, *K. Seto* 21626, 21627 (OSA28426, 28427). Aichi Pref.: Toyoake-shi, 8 Sep 1968, *S. Hamashima s.n.* (OSA185419). Shiga Pref.: Koka-cho, 29 Aug 2001, *K. Seto* 55464 (OSA161116); Makino-cho, 30 Aug 1966, *T. Kodama* 10864 (OSA3472). Kyoto Pref.: Miyazu-shi, 14 Jun 2001, *S. Fujii et al.* 8624 (OSA206370), 15 Sep 1995, *K. Seto* 44829 (OSA101400–101402). Nara Pref.: 9 Aug 1948, *M. Hutoh* 1084 (OSA198806); Nara-shi, 26 Sep 1965, *K. Seto* 14928 (OSA1743), 9 Jun 1963, *K. Seto* 12293 (OSA198809); Tenri-shi, 3 Sep 1999, *N. Morimoto* 4482 (OSA244026), 26 Sep 1997, *K. Seto* 48119 (OSA104917); Kitakatsuragi-gun, 1 Aug 1951, *T. Kodama s.n.* (OSA198808); Soni-mura, 24 Aug 2011, *N. Morimoto* 11924 (OSA188309); Tsukigase-mura, 14 Jun 2003, *K. Seto* 59208 (OSA165643). Osaka Pref.: Izumi-shi, 8 Sep 1985, *C. Shimizu s.n.* (OSA204544), 13 Oct 1983, *C. Shimizu s.n.* (OSA204561); Sakai-shi, 20 Aug 1933, *O. Kishimoto* 1083 (OSA); Shijonawate-shi, 26 Aug 1999, *M. Tanaka s.n.* (OSA148585), Aug 1931, *Kira s.n.* (OSA204041);

Shijonawate-cho, 24 Sep 1963, *K. Seto 12974* (OSA); 24 Sep 1963, *M. Kuwashima s.n.* (OSA); Senpoku-gun, 22 Sep 1960, *K. Seto 10407* (OSA58620). Mie Pref.: Shimagahara-mura, 23 Sep 1999, *K. Seto 51559* (OSA156483); Yokkaichi-shi, 2 Aug 1951, *N. Yasui s.n.* (OSA); 13 Aug 1950, *N. Yasui s.n.* (OSA); Ueno-shi, 21 Aug 2003, *K. Seto 59676* (OSA), 3 Oct 1999, *S. Fujii 7182* (OSA201287); Nabari-shi, 7 Aug 1992, *K. Seto 38848–38851* (OSA93981–93984). Hyogo Pref.: Kumobemura, 1 Jul 1928, *S. Yonezawa s.n.* (OSA180470); Takarazuka-shi, 13 Sep 1998, *K. Seto 49823* (OSA152575); 14 Sep 1997, *K. Seto 48062* (OSA150060). Wakayama Pref.: Wakayama-shi, 19 Sep 2000, *S. Fujii 8278* (OSA203436); Ono-shi, 17 May 2016, *Y. Kadono s.n.* (NGU4481–4483), 30 Sep 1998, *T. Fujii 10508* (OSA210073); Kato-shi, 11 Oct 2014, *T. Shiga 8299* (NGU2775). Hiroshima Pref.: Higashihiroshima-shi, 22 Sep 2013, *T. Shiga 7594* (NGU299). Kagawa Pref.: Mannouchi, 19 Aug 2016, *T. Shiga et al. s.n.* (NGU4125, 4488–4490). Kumamoto Pref.: Arao-shi, 10 Aug 1934, *H. Arao 692* (Herb. of Kumamoto Pref. Matsubase Store House = NB, 21-000681), 17 Oct 1933, *H. Arao 694, 695* (NB21-000683, 000684), 18 Sep 1932, *H. Arao 696, 697* (NB21-000685, 000686); Minamioguni-machi, 18 Jul 1993, *T. Maeda s.n.* (NB21-114265), *H. Nobukuni 4416* (NB21-006292); Ashikita, 1953, *M. Maeda s.n.* (NB21-050593); Kuma-mura, 16 Aug 1917, *S. Tokunaga s.n.* (NB21-050597); Aso-shi, 30 Sep 1906, *H. Kozuma s.n.* (NB21-050594).

Key to the *Sagittaria* species in Korea

1. Leaves linear to spatulate; carpellate flowers sessile *S. pygmaea* 올미
1. Leaves sagittate; carpellate flowers subsessile or pedicellate.
 2. Stolon absent; tubercles present in leaf axils; apex of basal lobes obtuse; basal lobes usually shorter than the middle lobe; scapes taller than the leaves *S. aginashi* 보풀
 2. Stolons with terminal tubers; tubercle absent; apex of basal lobes acute; basal lobes usually longer than the middle lobe; scapes usually shorter than the leaves *S. trifolia* 벚풀

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