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Systematic study of Korean *Asparagus* L. based on morphology and nuclear ITS sequences

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외부형태와 ITS 염기서열에 기초한 한국산 비짜루속 식물의 분류학적 고찰

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ABSTRACT : Morphological and geographical examinations as well as phylogenetic analyses using ITS sequences were performed for *Asparagus* L. in Korea. A total of five species of *Asparagus* were confirmed to be distributed in South Korea. The shape of cladophylls, length of pedicels, and shape of perianth were considered to be important characteristics for the identification of Koran *Asparagus* species. A monophyly of each species was evident in the ITS phylogenetic trees in which multiple accessions (5 to 24, depending on species) represented each of the five Korean species. *A. rigidulus* Nakai, once considered conspecific to *A. schoberioides* Kunth, formed a distinct lineage in the ITS trees. Pedicels of *A. rigidulus*, which is distributed mainly in coastal areas, were about two times longer than those of *A. schoberioides* occurring in inland areas, suggesting that they should be treated as distinct taxa.

Keywords: *Asparagus*, *A. davuricus*, *A. rigidulus*, *A. schoberioides*, ITS phylogeny

적 요: 한국산 *Asparagus* L. (비짜루속)을 대상으로 외부형태 형질을 재검토하고 ITS 염기서열을 사용한 계통분석을 실시하였다. 외부형태를 분석한 결과 엽상경의 형태, 소화경의 길이, 화피의 형태 등이 종을 식별하는 데 중요한 형질이었으며, 남한에는 최근에 분포가 확인된 *A. davuricus* Fisch. and Link (망적천문동)을 포함하여 총 다섯 종의 *Asparagus*가 자생하는 것으로 확인되었다. 각 분류군별로 5 내지 24 개체를 포함하여 수행한 ITS 염기서열 계통분석에서 각 분류군은 모두 단계통군을 이루었다. 특히 *A. schoberioides* Kunth (비짜루)의 이명으로 처리되고 있는 *A. rigidulus* Nakai (노간주비짜루)는 *A. shoberioides*와 8개의 뉴클레오티드 자리에서 차이를 보이면서 독자적인 단계통군을 형성하였다. 독도, 제주도 및 중남부 해안에 주로 자라는 *A. rigidulus*의 소화경은 내륙에 분포하는 *A. schoberioides*에 비해 약 2배 길었다. 두 종의 형태적, 지리적, 계통유전학적 차이는 *A. rigidulus*를 *A. schoberioides*와 다른 독립된 종으로 인정해야 함을 말해준다.

주요어: 비짜루속, 망적천문동, 노간주비짜루, 비짜루, ITS 분자계통

Asparagus L. (*Asparagaceae*) is comprised of about 160 species distributed in Europe, Asia, Africa, and Australia (Kubitzki and Rudall, 1998). Among the total number of species, 35 species occur in NE Asia (Il'in, 1968; Ohwi, 1972; Chen and Tamaian, 2000). Currently, *Asparagus* is classified into three subgenera: subgen. *Asparagus*, with dioecious taxa is distributed mainly throughout Asia; subgen. *Myrsiphyllum* Willd. and subgen. *Protasparagus* Oberm., both with hermaphroditic taxa occur in Africa (Clifford and Conran, 1987). Although both the male and female flowers of the dioecious species in subgen. *Asparagus* have pistil and stamen

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in their early developmental stage, sexual differentiation occurs by the selective abortion of the pistil or stamen in the male and female flowers, respectively (Dellaporta and Calderon-Urrea, 1933; Galli et al., 1993). Thus subgen. *Asparagus* is considered to be derived from a hermaphroditic ancestor (Clifford and Conran, 1987).

Research on Korean *Asparagus* plants was initiated by Palibin (1901) and Komarov (1901) who reported *A. schoberioides* Kunth and *A. oligoclonos* Maxim., respectively. Yabe (1903) reported *A. lucidus* Lindle. (= *A. cochinchinensis* (Lour.) Merr.) and Nakai (1913) reported *A. rigidulus* Nakai, *A. tamaboki* Yatabe, *A. davuricus* Fisch. ex Link. Nakai (1923) newly described *A. verrucosus* Nakai from Sorae (Incheon) in southern Korea, and Kitagawa (1939) reported *A. brachyphyllus* Turz. in northern Korea. These two species, however, were considered conspecific and were merged under the name of *A. brachyphyllus* (Noda, 1971). Ohwi (1972) treated *A. tamaboki* and *A. rigidulus* as a synonym of *A. oligoclonos* and *A. schoberioides*, respectively. It was evident that *A. tamaboki* was a male plant of *A. oligoclonos* having the rudimentary ovary (Makino, 1905). However, *A. rigidulus* was merged into *A. schoberioides* for reasons which are unknown (Ohwi, 1972).

Recently, Lee (2007) recognized only three native species of *Asparagus* in Korea: *A. cochinchinensis*, *A. oligoclonos*, and *A. schoberioides*. Reexamination on the herbarium specimens deposited in several different herbaria, however, revealed that *A. davuricus*, once known to occur only in North Korea, was also distributed in South Korea (Gyeonggi-do, Chungcheonnam-do, Gangwon-do, Gyeonsangbuk-do), suggesting the need for reexamination of the species diversity and distribution of Korean *Asparagus*. In addition, the taxonomic entity of *A. rigidulus*, which has been considered conspecific to *A. schoberioides* (Ohwi, 1972) needs further examination, since the taxonomic treatment was performed without specific reasons.

There have been comparative anatomy (Kim and Lee, 1985) and karyological studies (Kim and Oh, 1985) for three species of *Asparagus* in Korea. Although these studies provided some basic information for the identification of the examined species, major systematic issues, especially phylogenetic relationship and geographic distribution of the species were not addressed. There have also been comparative studies on the genus *Asparagus* using the RFLP (Restriction Fragment Length Polymorphism) of nuclear ribosomal ITS (Internal Transcribed Spacer) region (Stajner et al., 2002) and plastid DNA (Lee et al., 1997). However, these studies were mainly about the selection of close relatives of *A. officinalis* L. to produce useful cultivar through hybridization process, and no Korean taxa were included in the analyses. A phylogenetic study based on DNA sequences of the plastid genome for 26 taxa (including some Korean species) of *Asparagus* has been undertaken by Fukuda et al. (2005). However, they chose *pet*B intron and *pet*D-*rpo*A IGS (intergenic spacer) regions which show extremely low sequence variation, resulting in no resolution for the species relationship and only limited discussion on the subgeneric relationship.

This study aimed to reexamine the distribution and morphological characters of Korean *Asparagus* taxa, and to discuss their phylogenetic relationships using the DNA sequences of nuclear ribosomal ITS regions.

Materials and Methods

A total of one hundred specimens, obtained either from several herbaria (KWNU, KNU, KH, IUI, TI) or from the field collection, were examined for morphological characters of all Korean *Asparagus* species except for *A. brachyphyllus*, which is known to be distributed mainly in North Korea (Appendix I). Multiple accessions representing each species (5–24 individuals depending on species) were employed for molecular phylogenetic analyses. The collection data and voucher information are provided in Table 1. A voucher specimen is missing for some accessions collected from a population with very limited number of individuals.

Morphological characters which have been thought to be important for the classification of *Asparagus* (Lee, 2007) were carefully examined for the selected taxa. Several diagnostic characters, including the type of roots, the shape and length of cladophylls, the length of pedicels, the shape of perianth and the length of tepals, were chosen for species comparison. The same position or part of each organ was measured to avoid variation due to varying developmental stages.

Total DNA was isolated from fresh leaves or dried herbarium specimens using the DNeasy plant mini kit according to the manufacturer's manual (Qiagen, Germany). The ITS regions were amplified and sequenced using primers designed by White et al. (1990) except ITS1, which differed by the two underlined bases (5'-GGAAGGAGAAGTCGTAACAAGG-3'). PCR amplifications were carried out in a total reaction volume of 50 μL containing 5 × GoTaq Flexi buffer 10 μL, 25 mM $MgCl_2$ 4 μL, 2.5 mM dNTPs 4 μL, forward and reverse primers each 0.5 μL, 5 units/μL Taq polymerase 0.25 μL, 10 to 20 ng template DNA, and distilled water to the final volume. The thermocycling profile consisted of an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 1 min at 95°C, 1 min at 55.5°C, 1 min at 72°C, and a final extension step of

Table 1. Voucher information and distribution of *Asparagus* taxa included in this study. Province abbreviations: CB (Chungcheongbuk-do), CN (Chungcheongnam-do), GB (Gyeongsangbuk-do), GG (Gyeonggi-do), GN (Gyeongsangnam-do), GW (Gangwon-do), JB (Jeollabuk-do), JJ (Jeju-do), JN (Jeollanam-do).

Accession No.	Voucher information	Locality	GenBank No.
Asparagus cochinchinensis (01)	*S.H. Cho et al. ChoSH-001* (HHU)	JN, Naju-gun	JN171595
A. cochinchinensis (02)	*S.H. Cho et al. ChoSH-002* (HHU)	JN, Hwasun-gun	JN171596
A. cochinchinensis (03)	no voucher	JN, Hwasun-gun	JN171597
A. cochinchinensis (04)	no voucher	JN, Hwasun-gun	JN171598
A. cochinchinensis (05)	no voucher	JN, Hwasun-gun	JN171599
A. davuricus (01)	*S.H. Cho and Y.I Kim ChoSH-006* (HHU)	CN, Taean-gun	JN171600
A. davuricus (02)	*S.H. Cho and Y.I Kim ChoSH-007* (HHU)	CN, Taean-gun	JN171601
A. davuricus (03)	*S.H. Cho and Y.I Kim ChoSH-008* (HHU)	CN, Taean-gun	JN171602
A. davuricus (04)	no voucher	CN, Taean-gun	JN171603
A. davuricus (05)	*S.H. Cho and Y.I Kim ChoSH-009* (HHU)	GG, Hwaseong-si	JN171604
A. davuricus (06)	*S.H. Cho and Y.I Kim ChoSH-010* (HHU)	GG, Hwaseong-si	JN171605
A. davuricus (07)	*S.H. Cho et al. ChoSH-020* (HHU)	GW, Samcheok-si	JN171606
A. davuricus (08)	*S.H. Cho et al. ChoSH-025* (HHU)	CN, Boryoeng-si	JN171607
A. davuricus (09)	no voucher	CN, Boryoeng-si	JN171608
A. davuricus (10)	*S.H. Cho et al. ChoSH-027* (HHU)	CN, Boryoeng-si	JN171609
A. falcatus	*C.H. Oh Yeomiji-398* (KH)	Botanical Garden Yeomiji	JN171610
A. oligoclonos (01)	*S.H. Cho and Y.I Kim ChoSH-028* (HHU)	GG, Hwaseong-si	JN171611
A. oligoclonos (02)	*S.H. Cho and Y.I Kim ChoSH-029* (HHU)	GG, Hwaseong-si	JN171612
A. oligoclonos (03)	no voucher	GG, Hwaseong-si	JN171613
A. oligoclonos (04)	no voucher	GG, Hwaseong-si	JN171614
A. oligoclonos (05)	*S.H. Cho et al. ChoSH-030* (HHU)	GW, Pyeongchnag-gun	JN171615
A. oligoclonos (06)	*S.H. Cho et al. ChoSH-031* (HHU)	GW, Yeongwol-gun	JN171616
A. oligoclonos (07)	*S.H. Cho et al. ChoSH-032* (HHU)	GW, Yeongwol-gun	JN171617
A. rigidulus (01)	*S.H. Cho et al. ChoSH-039* (HHU)	GN, Sacheon-si	JN171618
A. rigidulus (02)	*S.H. Cho et al. ChoSH-040* (HHU)	JJ, Jeju-si	JN171619
A. rigidulus (03)	no voucher	JJ, Seogwipo-si	JN171620
A. rigidulus (04)	*S.H. Cho et al. ChoSH-041* (HHU)	JJ, Seogwipo-si	JN171621
A. rigidulus (05)	no voucher	JJ, Seogwipo-si	JN171622
A. rigidulus (06)	no voucher	JJ, Seogwipo-si	JN171623
A. rigidulus (07)	*S.H. Cho et al. ChoSH-044* (HHU)	JJ, Seogwipo-si	JN171624
A. rigidulus (08)	*S.H. Cho et al. ChoSH-045* (HHU)	JJ, Jeju-si	JN171625
A. rigidulus (09)	*S.H. Cho et al. ChoSH-046* (HHU)	JJ, Jeju-si	JN171626
A. rigidulus (10)	*S.H. Cho et al. ChoSH-047* (HHU)	JJ, Jeju-si	JN171627
A. rigidulus (11)	*S.H. Cho et al. ChoSH-048* (HHU)	JN, Haenam-gun	JN171628
A. rigidulus (12)	*S.H. Cho et al. ChoSH-049* (HHU)	CN, Taean-gun	JN171629
A. rigidulus (13)	no voucher	CN, Taean-gun	JN171630
A. rigidulus (14)	*S.H. Cho et al. ChoSH-050* (HHU)	JJ, Jeju-si	JN171631
A. rigidulus (15)	*S.H. Cho et al. ChoSH-051* (HHU)	JJ, Jeju-si	JN171632
A. rigidulus (16)	*S.H. Cho et al. ChoSH-052* (HHU)	GB, Uljin-gun	JN171633
A. rigidulus (17)	*S.H. Cho et al. ChoSH-053* (HHU)	GB, Uljin-gun	JN171634
A. rigidulus (18)	*Pak and Lee* 8349 (KNU)	GB, Ulleung-gun (Dok-do)	JN171635
A. rigidulus (19)	*Pak and Lee* 8329 (KNU)	GB, Ulleung-gun (Dok-do)	JN171636
A. rigidulus (20)	*S.H. Cho and J.H Lee ChoSH-168* (HHU)	JN, Shinan-gun (Gageo-do)	JN171637
A. rigidulus (21)	*S.H. Cho and J.H Lee ChoSH-170* (HHU)	JN, Shinan-gun (Gageo-do)	JN171638
A. rigidulus (22)	no voucher	JN, Shinan-gun (Gageo-do)	JN171639
A. rigidulus (23)	*S.H. Cho and J.H Lee ChoSH-196* (HHU)	JN, Shinan-gun (Heuksan-do)	JN171640
A. rigidulus (24)	*S.H. Cho and J.H Lee ChoSH-197* (HHU)	JN, Shinan-gun (Heuksan-do)	JN171641

Table 1. Continued.

Accession No.	Voucher information	Locality	GenBank No.
A. schoberioides (01)	*S.H. Cho and N.R Kim ChoSH-127* (HHU)	GW, Samcheok-si	JN171642
A. schoberioides (02)	*S.H. Cho and N.R Kim ChoSH-054* (HHU)	GW, Samcheok-si	JN171643
A. schoberioides (03)	*S.H. Cho and N.R Kim ChoSH-055* (HHU)	GW, Samcheok-si	JN171644
A. schoberioides (04)	*C.G. Lee and J.H Lee ChoSH-056* (HHU)	GW, Chuncheon-si	JN171645
A. schoberioides (05)	no voucher	CB, Danyang-gun	JN171646
A. schoberioides (06)	no voucher	GB, Cheongsong-gun	JN171647
A. schoberioides (07)	*M.S. Choi et al. 902028* (KH)	GB, Bonghwa-gun	JN171648
A. schoberioides (08)	*Kim et al. KHB1182720* (KH)	JB, Muju-gun	JN171649
A. schoberioides (09)	*Kim et al. KHB1182721* (KH)	JB, Muju-gun	JN171650
A. schoberioides (10)	*H.-J. Choi et al. CHJ60036* (KH)	CN, Cheonan-si	JN171651
A. schoberioides (11)	*E.-H. Jung & H. K. Moon K0525-031* (KH)	GW, Hwacheon-gun	JN171652
A. schoberioides (12)	*B.O. Oh et al. CBU-070023* (KH)	GB, Bonghwa-gun	JN171653
A. schoberioides (13)	*B.O. Oh et al. CBU-070148* (KH)	CB, Cheongwon-gun	JN171654
A. schoberioides (14)	*J.H. Kim et al. KHB1183679* (KH)	GG, Pocheon-si	JN171655
A. schoberioides (15)	*Kim et al. KHB1182722* (KH)	CB, Gyeosan-gun	JN171656
A. schoberioides (16)	*S.H. Cho et al. ChoSH-167* (HHU)	GG, Gapyeong-gun	JN171657
A. schoberioides (17)	*W.K. Paik VP-KB-377063-0206* (KB)	GG, Namyangju-si	JN171658
A. schoberioides (18)	*Hui KIM HKIM3145* (KB)	JN, Gokseong-gun	JN171659
A. schoberioides (19)	*S.H. Cho et al. ChoSH-185* (HHU)	JJ, Seogwipo-si	JN171660
A. schoberioides (20)	*S.H. Cho et al. ChoSH-190* (HHU)	JJ, Jeju-si	JN171661
A. schoberioides (21)	*S.H. Cho et al. ChoSH-192* (HHU)	JJ, Jeju-si	JN171662
A. schoberioides (22)	*S.H. Cho et al. ChoSH-194* (HHU)	JJ, Jeju-si	JN171663

10 min at 72°C (Applied Biosystems Gene Amp. PCR system 9700). The PCR products were purified with a QIAqiuck PCR purification kit according to the manufacturer's protocol (Qiagen, Germany). Purified double-stranded PCR products were used for determining the DNA sequences of ITS regions using 3730xl DNA analyzer (Automatic Biosystems). All sequences were deposited in GenBank (Table 1).

The DNA sequences were aligned with CLUSTAL X (Thompson et al., 1997). The alignment was further examined and slightly edited using MacClade 4.03 PPC (analysis of phylogeny and character evolution. 4.03 PPC; Maddison and Maddison, 2002) as necessary. Gaps introduced from the alignment were treated as missing characters in subsequent analyses. Both Neighbor Joining (NJ) and Maximum Parsimony (MP) analyses were conducted using PAUP* 4.0 (Phylogenetic Analysis Using Parsimony, version 4.0b10, Swofford, 2001). For the NJ tree Kimura's 2 parameter method (Kimura, 1980) was selected for estimating the genetic distance among the OTUs. The maximum parsimony analyses were conducted using heuristic searches with the MULTREES option, 10 random entries of taxa, and TBR (Tree Bisection and Reconnection) branch swapping. Bootstrap analyses (Felsenstein, 1985) with 1000 replicates were conducted to evaluate the degree of support for given clades of NJ and MP trees (PAUP* ver. 4.0b10, Swofford 2001). The trees were rooted using *Asparagus falcatus* L. as an outgroup (Fukuda et al., 2005).

Results

1. Distribution of *Asparagus* in South Korea

The distribution of *Asparagus* species in South Korea is shown in Fig. 1. *A. davuricus* which had been known only to occur in China, Russia, Mongol, and North Korea was confirmed to be widely distributed along the east and west coasts of South Korea (Fig. 1). *A. schoberioides* is distributed in inland areas, while its closely related species *A. rigidulus* has been found mostly along coastal areas including Dok-do (Fig. 1). *A. cochinchinensis* was observed mainly in south-western part of South Korea, whereas *A. oligoclonos* is widely distributed throughout the country, though not in Jeju-do or Ulleung island.

2. Morphological characteristics of Korean *Asparagus*

The morphological characteristics, including the shape,

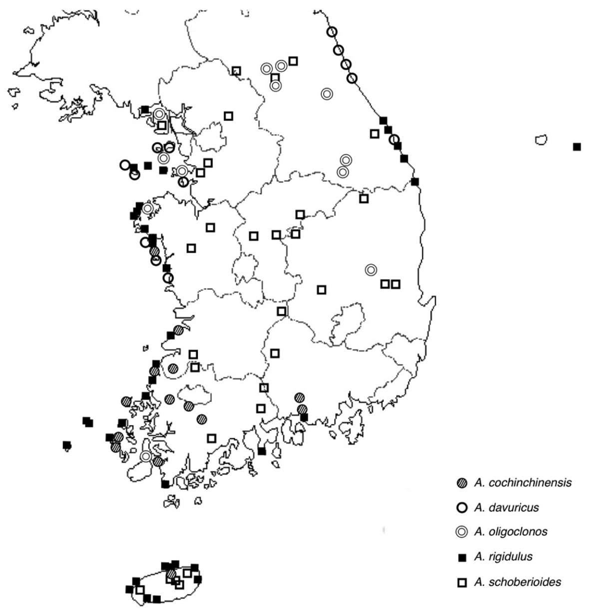

Fig. 1. Distribution of *Asparagus* species in South Korea.

average length, standard deviation, and maximum-minimum measured values of major parts of the examined species are shown in Table 2.

The root was terete in all examined species except for *A. cochinchinensis* which had a fusiform root. In terms of the shape of cladophylls, two types were observed: straight type (*A. oligoclonos*, *A. davuricus*) and falcate type (*A. cochinchinensis*, *A. schoberioides*, *A. rigidulus*). The cladophyll was longest in *A. oligoclonos* which was followed by *A. davuricus*, *A. rigidulus*, *A. schoberioides*, and *A. cochinchinensis*.

The number of cladophyll in the fascicle varied depending on the taxa. *A. oligoclonos* had 3–6 cladophylls while *A. davuricus* generally had 1 or 2–5 cladophylls, yet the number was extremely variable depending on the individual. *A. cochinchinensis* had 2–3 cladophylls and *A. rigidulus* generally had 2–5 cladophylls. *A. schoberioides* mostly had 2–3 cladophylls, but more rarely 4–5 cladophylls were found in fascicles of this species.

The length of pedicels exhibited significant differences among the examined taxa. Pedicels of *Asparagus oligoclonos* were ca. 17.31 mm long while those of *A. schoberioides* were ca. 0.87 mm long. The pedicel length of *A. rigidulus* was ca. 1.61 mm, about two times longer that of *A. schoberioides*. *A. davuricus* and *A. cochinchinensis* had pedicel lengths of ca. 4.47 mm and ca. 3.96 mm, respectively. No significant length difference was observed between pedicels of male and female flowers in each species.

The flowers of the examined taxa were either tubular or campanulate. In *A. oligoclonos* and *A. davuricus* the male flowers were tubular, while the female flowers were campanulate with reflexed tepal tips. Both male and female flowers of *A. rigidulus* and *A. schoberioides* were campanulate and the tip of the tepal was straight. *A. cochinchinensis* has

Table 2. Morphological characteristics of the Korean *Asparagus* species examined in the present study.

		A. oligoclonos	*A. davuricus*	*A. cochinchinensis*	*A. schoberioides*	*A. rigidulus*
Plant height (cm)		57–150	33–120	71	49–100	57–190
Shape of root		terete	terete	fusiform	terete	terete
Shape of cladophyll		straight	straight	falcate	falcate	falcate
Cladophyll length (mm)		27.99±8.82 (12–58)*	25.62±11.02 (4–60)	12.75±7.37 (4–33)	15.38±5.65 (6–35)	17.96±6.84 (6–55)
Number of cladophyll in a fascicle		3–6	1–5	2–3	2–3 [4–5]	2–4 [5]
Pedicel length (mm)		17.31±.05 (8.1–25)	4.47±0.99 (1.99–7.04)	3.96±0.81 (2.8–5.95)	0.87±0.23 (0.6–1.2)	1.61±0.35 (1.2–2.73)
Number of flower		2, rarely 1	2, rarely 1 or 3–4	2, rarely 1 or 3	2–4, rarely 1	2–4
Shape of flower	male	tubular	tubular	widely campanulate	campanulate	campanulate
	female	campanulate	campanulate	no data	campanulate	campanulate
Shape of tepal		with reflexed tip	with reflexed tip	spreading	with straight tip	with straight tip
Tepal length (mm)	male	7.31±1.35 (5–10)	5.43±0.39 (4.67–6.2)	3.00±0.53 (2.55–3.87)	2.60±0.46 (1.80–3.13)	3.08±0.29 (2.54–4.14)
	female	7.01±0.70 (6–8)	2.84±0.50 (2.1–3.65)	no data	3.02±0.02 (3.00–3.04)	2.42±0.10 (2.31–2.50)
Fruit length (mm)		8–10	5–8	6–7	6–7	6–7
Number of seed per fruit		1–5	1–4	1	1–2	1–2

*Minimum and maximum lengths are in parentheses.

Table 3. ITS sequence variations observed from the accessions of *Asparagus schoberioides* and *A. rigidulus*. A dot indicates that the sequence is the same as the first accession listed. Refer to Table 1 for information of each accession. (Nucleotide ambiguity codes : R = A/G, W = A/T, Y = C/T, K = G/T, M = A/C, S = C/G).

Accession	Nucleotide position							
	9	35	38	154	172	183	394	413
A. schoberioides (01)	G	C	C	A	A	G	T	T
A. schoberioides (02)	.	.	.	.	.	.	.	.
A. schoberioides (03)	.	.	.	.	.	.	.	.
A. schoberioides (04)	.	.	.	.	.	.	.	.
A. schoberioides (05)	.	.	.	.	.	.	.	.
A. schoberioides (06)	.	.	.	.	.	.	.	.
A. schoberioides (07)	.	.	.	.	.	.	.	.
A. schoberioides (08)	.	.	.	.	.	.	.	.
A. schoberioides (09)	.	.	.	.	.	.	.	.
A. schoberioides (10)	.	.	.	.	.	.	.	.
A. schoberioides (11)	.	.	.	.	.	.	.	.
A. schoberioides (12)	.	.	.	.	.	.	.	.
A. schoberioides (13)	.	.	.	.	.	.	.	.
A. schoberioides (14)	.	.	.	.	.	.	.	.
A. schoberioides (15)	.	.	.	.	.	.	.	.
A. schoberioides (16)	.	.	.	.	.	.	.	.
A. schoberioides (17)	.	.	.	.	.	.	.	.
A. schoberioides (18)	.	.	.	.	.	.	.	.
A. schoberioides (19)	R	.	.	.	.	.	.	.
A. schoberioides (20)	R	.	.	.	.	.	.	.
A. schoberioides (21)	R	.	.	.	.	.	.	.
A. schoberioides (22)	.	.	.	.	.	.	.	.
A. rigidulus (01)	A	T	T	C	T	A	G	C
A. rigidulus (02)	A	T	T	C	T	A	G	C
A. rigidulus (03)	A	T	T	C	T	A	G	C
A. rigidulus (04)	A	T	T	C	T	A	G	C
A. rigidulus (05)	A	T	T	C	T	A	G	C
A. rigidulus (06)	A	T	T	C	T	A	G	C
A. rigidulus (07)	A	T	T	C	T	A	G	C
A. rigidulus (08)	A	T	T	C	T	A	G	C
A. rigidulus (09)	A	T	T	C	T	A	G	C
A. rigidulus (10)	A	T	T	C	T	A	G	C
A. rigidulus (11)	A	T	T	C	T	A	G	C
A. rigidulus (12)	A	T	T	C	T	A	G	C
A. rigidulus (13)	A	T	T	C	T	A	G	C
A. rigidulus (14)	A	T	Y	C	T	R	G	Y
A. rigidulus (15)	A	T	T	C	T	A	G	C
A. rigidulus (16)	A	T	T	C	T	A	G	C
A. rigidulus (17)	A	T	T	C	T	A	G	C
A. rigidulus (18)	A	T	T	C	T	A	G	C
A. rigidulus (19)	A	T	T	C	T	A	G	C

Table 3. Continued.

Accession	Nucleotide position							
	9	35	38	154	172	183	394	413
A. rigidulus (20)	A	T	T	C	T	A	G	C
A. rigidulus (21)	A	T	T	C	T	A	G	C
A. rigidulus (22)	A	T	T	C	T	A	G	C
A. rigidulus (23)	A	T	Y	C	T	A	G	C
A. rigidulus (24)	A	T	T	C	T	A	G	C

widely campanulate flowers in male individuals with the tepals spread fully. We were unable to observe or collect a specimen with pistillate flower in the present study.

The length of tepals was variable depending on species and differed between male and female flowers in each species. The tepal lengths of *A. oligoclonos* were ca. 7.31 and 7.01 mm in male and female flowers, respectively. The tepal length of *A. davuricus* was ca. 5.43 mm in male flowers, while that females was ca. 2.84 mm. *A. cochinchinensis* has a tepal length of ca. 3.00 mm in male flowers. In *A. schoberioides* the tepal length of male flowers was shorter (ca. 2.60 mm) than those of female flowers (ca. 3.02 mm), whereas the tepals of male flowers in *A. rigidulus* were longer than those of female flowers.

The fruit of *A. oligoclonos* was the longest, followed by *A. cochinchinensis*, *A. schoberioides*, *A. rigidulus*, and that of *A. davuricus* was the shortest. The number of seeds was variable within each species (1–2, 1–4, 1–5 depending on species) except for *A. cochinchinensis*, which consistently had only one seed.

The following key distinguishes *Asparagus* examined in this study.

Key to *Asparagus* species distributed in South Korea

1. Cladophylls straight
  2. Pedicels longer than 8.1 mm, fruit diam. 8–10 mm .... *A. oligoclonos* 방울비짜루
  2. Pedicels shorter than 7.1 mm, fruit diam. 5–8 mm .... *A. davuricus* 망적천문동
1. Cladophylls falcate
  3. Woody vine, pedicels 3.0–6.8 mm long, tepals of female flower reflexed .... *A. cochinchinensis* 천문동
  3. Perennial herb, pedicels 0.6–2.7 mm long, tepals of female flower straight
    4. Pedicels ca. 0.87 mm long .... *A. schoberioides* 비짜루
    4. Pedicels ca. 1.61 mm long .... *A. rigidulus* 노간주비짜루

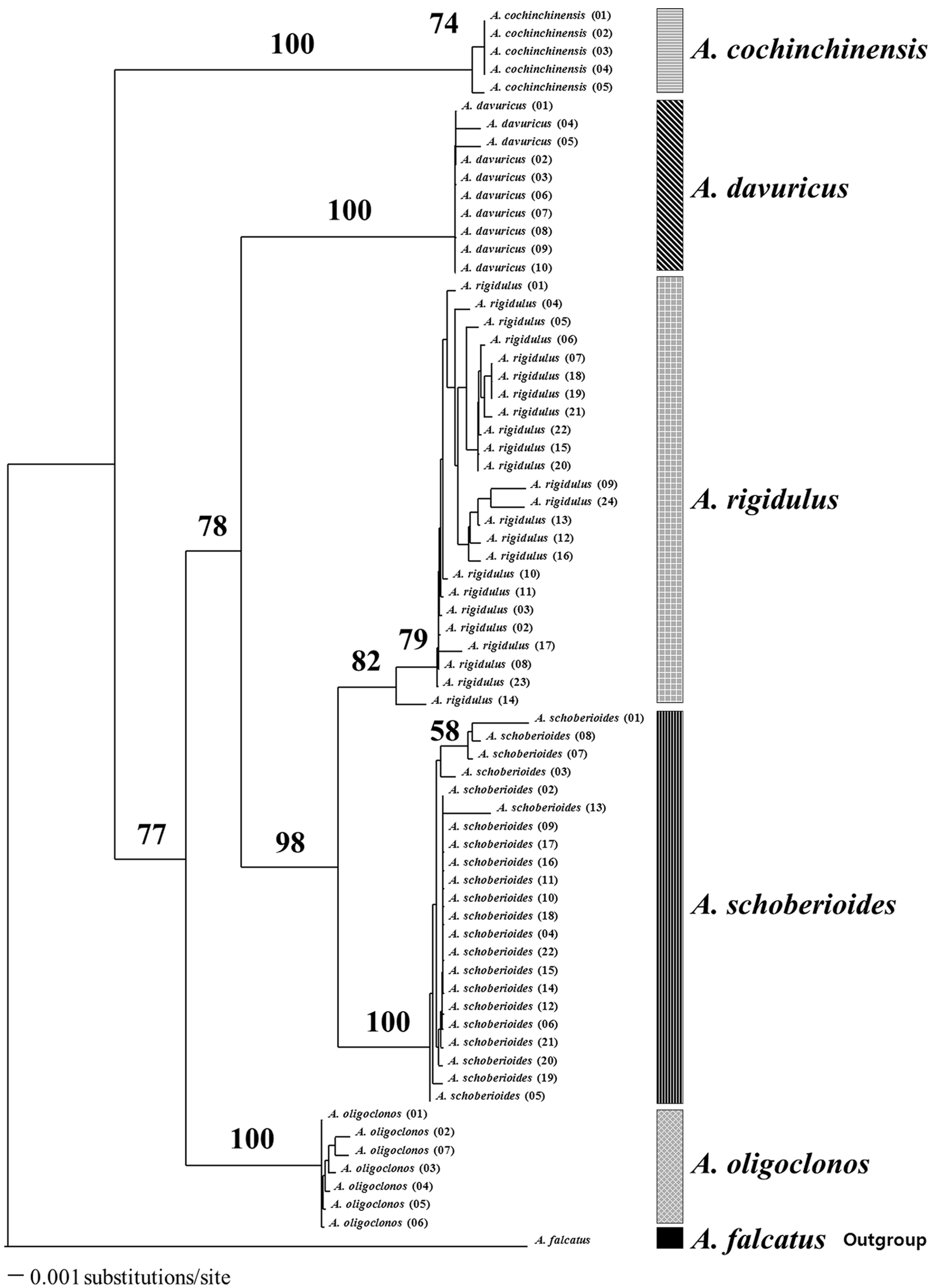

Fig. 2. Phylogenetic tree of Korean *Asparagus* reconstructed by the neighbor-joining method. Bootstrap supports are indicated on the branches. Refer to Table 1 for information of each accession.

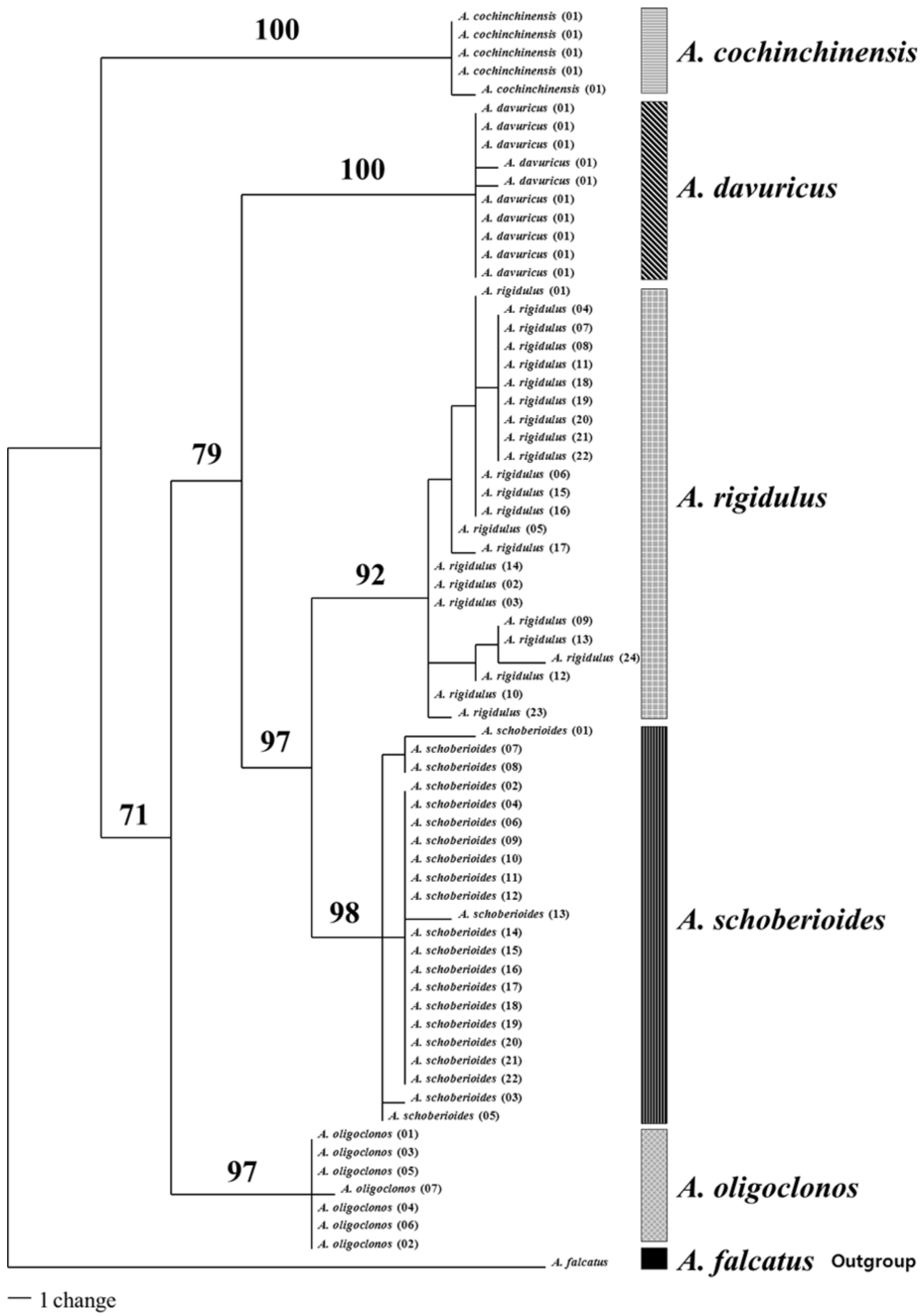

Fig. 3. A strict consensus tree of 546 equally parsimonious trees based on ITS sequences of Korean *Asparagus*. Bootstrap supports are indicated on the branches. Refer to Table 1 for information of each accession.

3. ITS sequences and phylogeny

The lengths of ITS regions (including 5.8S) of the examined taxa were 619 bp for *Asparagus cochinchinensis*, 621 bp for *A. davuricus*, 622 bp for *A. schoberioides* and *A. rigidulus*, and 625 bp for *A. oligoclonos*. *A. schoberioides* and *A. rigidulus*, which are often treated conspecific, exhibited differences in a total of eight nucleotide sites. Of the eight sites, a weak polymorphism was observed in four sites, while the rest of the sites were unambiguous (Table 3).

Phylogenetic trees based on the aligned ITS sequences exhibited identical topology in neighbor-joining (NJ) and the maximum-parsimony (MP) trees, in which all examined taxa with multiple accessions formed clades supported by high bootstrap values (Figs. 2, 3). In MP analysis, a total of 546 equally parsimonious trees with the length of 97, CI (excluding uninformative characters) of 0.887, and RI of 0.979 was obtained. In both of the MP and NJ trees, *A. cochinchinensis* was identified as basal clade, followed by *A. oligoclonos* which showed a sister group relationship with the rest of the species. *A. schoberioides* and *A. rigidulus* formed a strong clade together, and then grouped with *A. davuricus* (Figs. 2, 3).

Discussion

1. Distribution and morphology of Korean *Asparagus*

This study provided updated information on the geographic distribution of *Asparagus* in Korea, especially for *Asparagus davuricus*, which has long been known to occur only in East Siberia, Far East, Mongolia, China, and North Korea. Although the species has been precisely referred to in the floristic literatures of neighboring countries including China (Chen and Tamaian, 2000), Russia (Il'in, 1968), North Korea (Im, 2000), it has been either missing from the major floristic works of South Korea (Lee, 1979; Lee, 2007) or only briefly mentioned (Lee, 1996), making the distribution of the species in South Korea questionable. This study clearly indicates that the distribution area of the species should be extended to the southern Korean Peninsula (coastal areas of Gyeonggi-do, Chungcheongnam-do, Gangwon-do, Gyeongsangbuk-do; Fig. 1).

The distribution of *A. brachyphyllus* in South Korea was not confirmed in the present study. We visited Sorae, the type locality of *A. verrucosus* (= *A. brachyphyllus*), but the original habitat seemed to be destroyed during the urbanization and expansion of Incheon city.

Morphological data indicates that the shape of the cladophyll (straight vs. falcate) is the primary character distinguishing Korean *Asparagus* (Table 2; also refer the key for the examined taxa). The morphological examination also revealed that the shape of the perianth and the length of pedicels and tepals (of female flowers) are important characteristics which can be used to distinguish between Korean *Asparagus* taxa. Two closely allied species *A. schoberioides* and *A. rigidulus* differed in the length of pedicels, supporting the taxonomic treatment of Nakai (1913), who noticed the length of pedicels in taxonomic delimitation. On the other hand, the length of the cladophyll, the position of articulation (not shown in Table 2), and the length of tepals in male flowers exhibited continuous variation (Table 2), which may be the reason why *A. davuricus* has been misidentified as *A. oligoclonos* in many herbarium specimens. Our data indicates that the lengths of pedicels and tepals (in female flowers) are the most important characteristics discerning differences between the two species (Table 2).

2. Taxonomic status of *Asparagus rigidulus*

This study provided important information for discussing the systematic issues pertaining Korean *Asparagus*, especially the taxonomic status of *Asparagus rigidulus*, which was described by Nakai (1913) but was later merged into *A. schoberioides* (Ohwi 1972). *A. rigidulus* is morphologically very similar to the more widely distributed species of *A. schoberioides*, except that the former species has slightly longer pedicels (*A. rigidulus* : 1.2–2.7 mm, *A. schoberioides* : 0.6–1.2 mm; Table 2) and a higher number of aerial shoots than the latter ones (personal observation). Considering the lack of distinct diagnostic characters, Ohwi (1972) treated *A. rigidulus* as a synonym of *A. schoberioides*. The molecular data was based on a large number of individuals (24 and 22 from *A. rigidulus* and *A. schoberioides*, respectively) collected from the whole distribution area in Korea, however, it suggested that they are well separated evolutionary lineages, supporting recognition of *A. rigidulus* as a distinct species within the genus *Asparagus*. In this study, the plant collection data also indicated that *A. rigidulus* occurred only in coastal areas of the Korean peninsula, Jeju-do, and Dok-do while *A. schoberioides* was distributed mainly in inland areas (Fig. 1). Despite the molecular and geographical differences between the two species, they exhibit very weak morphological differences, which suggest that *A. rigidulus* is either a cryptic taxon (Okuyama and Kato, 2009) or a recently diverged taxon from the common ancestor of *A. schoberioides* and *A. regidulus*. Additional collections of *A. rigidulus* from Japan are needed for a more robust conclusion to the evolution and taxonomy of the species.

3. Phylogenetic utility of ITS sequence

DNA sequences of ITS regions were very useful in the construction of phylogenetic trees for the examined taxa. All ITS regions of the examined accessions showed no severe within-individual polymorphism permitting direct sequencing without any cloning procedure. All individuals representing each taxon formed strongly supported clades, respectively (Figs. 2, 3). A total of 77 nucleotide sites observed from the four taxa *Asparagus cochinchinensis*, *A. davuricus, A. oligoclonos, A. schoberioides* (aligned sequence data not shown). This contrasts with the results from the examination of plastid DNA sequence data (*pet*B intron and *pet*D-*rpo*A intergenic spacer; Fukuda et al. 2005) in which extremely low variation (six sites were variable for the four taxa) was detected. Fukuda et al. (2005) postulated that an adaptive radiation was attributable to the low sequence variation in *Asparagus*. Recently, Kim and Kim (2011) showed that ITS sequence data were very useful when plastid DNA data fail to resolve phylogenetic relationships in *Chrysosplenium*. This study ascertains that the nuclear ribosomal ITS sequences, despite several drawbacks for phylogeny reconstruction (lvarez and Wendel, 2003), still produce insightful results in phylogenetic studies, provided that they are used carefully (Feliner and Rossell, 2007). Nuclear ribosomal ITS DNA sequence data with extended taxon sampling will provide significant implications for the phylogenetic relationship and character evolution of *Asparagus*.

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Appendix I. Herbarium specimens examined for morphology.

Asparagus cochinchinensis **(Lour.) Merr.**

Korea: *D.K. Lee s.n.* (specimen no. 14814) (IUI), *S.H. Cho et al. ChoSH-001* (HHU), *S.H. Cho et al. ChoSH-002* (HHU), *S.H. Park ParkSh51607* (KH), *W.C. Lee 0022932* (KWNU), *W.C. Lee 0022956* (KWNU), *Y.H. Cho WR-070530-127* (KH), *Y.H. Cho WR-070606-101* (KH).

Asparagus oligoclonos **Maxim.**

Korea: *B.G. Yoon 0022940* (KWNU), *B.H. Choi 1365* (IUI), *B.H. Choi et al. 5066* (IUI), *B.H. Choi et al. 177* (IUI), *B.H. Choi et al. 6217* (IUI), *B.H. Choi et al. 6220* (IUI), *B.H. Choi et al. 6221* (IUI), *B.H. Choi et al. 6224* (IUI), *J.S. Lee & S.K. Cho 883* (IUI), *J.S. Lee 5112* (IUI), *K.O. Yoo 0001062* (KWNU), *K.O. Yoo 0001063* (KWNU), *K.O. Yoo 058378* (KWNU), *K.O. Yoo 061205* (KWNU), *K.O. Yoo 061208* (KWNU), *K.O. Yoo 061210* (KWNU), *S.H. Cho & Y.I. Kim ChoSH-028* (HHU), *S.H. Cho & Y.I. Kim ChoSH-029* (HHU), *S.H. Cho et al. ChoSH-030* (HHU), *S.H. Cho et al. ChoSH-031* (HHU), *S.H. Cho et al. ChoSH-032* (HHU), *W.C. Lee 0022939* (KWNU), *W.C. Lee et al. 066494* (KWNU), *Y.M. Yang 82* (IUI).

Asparagus rigidulus **Nakai**

Korea: *B.H Choi & J.H. Lee 68056* (IUI), *B.H Choi & J.H. Lee 68912* (IUI), *B.H. Choi et al. 65025* (IUI), *B.H. Choi et al. 65026* (IUI), *B.H. Choi et al. s.n.* (specimen no. 035514) (IUI), *D.S. Ryu WR-070523-162* (KH), *E.S. Jeon 40595* (KH), *H.S. Cho s.n.* (specimen no, 039360) (IUI), *H.S. No s.n.* (specimen no. 5818) (IUI), *J.H. Lee 68126* (IUI), *M.K. Yoo s.n.* (specimen no. 0021) (IUI), *S.C. Park s.n.* (specimen no. 0022) (IUI), *S.C. Park s.n.* (specimen no. 15125) (IUI), *Seo 30827* (IUI), *S.H. Cho et al. ChoSH-039* (HHU), *S.H. Cho et al. ChoSH-040* (HHU), *S.H. Cho et al. ChoSH-041* (HHU), *S.H. Cho et al. ChoSH-044* (HHU), *S.H. Cho et al. ChoSH-045* (HHU), *S.H. Cho et al. ChoSH-046* (HHU), *S.H. Cho et al. ChoSH-047* (HHU), *S.H. Cho et al. ChoSH-048* (HHU), *S.H. Cho et al. ChoSH-049* (HHU), *S.H. Cho et al. ChoSH-050* (HHU), *S.H. Cho et al. ChoSH-051* (HHU), *S.H. Cho et al. ChoSH-052* (HHU), *S.H. Cho et al. ChoSH-053* (HHU), *S.H. Park 70459* (KH), *T. Nakai 1349* (TI), *T. Nakai 4874* (TI), *T. Nakai 10848* (TI), *T. Nakai 10849* (TI), *W.C. Lee 0022948* (KWNU), *W.C. Lee 0022960* (KWNU), *W.C. Lee 0022961* (KWNU), *W.C. Lee 0022963* (KWNU), *Y.H. Cho WR-070530-186* (KH), *Anonymous s.n.*-23 Jul 2003 (specimen no. 033732) (IUI).

Japan: *T. Nakai 1438* (TI), *T. Nakai 2625* (TI).

Asparagus schoberioides **Kunth**

Korea: *B.H. Choi et al. 230* (IUI), *B.H. Choi et al. 4712* (IUI), *B.H. Choi et al. s.n.* (specimen no. 032053) (IUI), *B.U. Oh CBU-070666* (KH), *B.U. Oh 060824-014* (KH), *B.U. Oh 060528-001* (KH), *B.U. Oh 040508-115* (KH), *E.K. Jeong s.n.* (specimen no. 033110) (IUI), *H.H. Hong 056098-a* (KH), *J.S. Lee 639* (IUI), *J.Y. Jung & S.G. Kwon s.n.* (specimen no. 15921) (IUI), *K.O. Yoo 058334* (KWNU), *S.G. Yang s.n.* (specimen no. 00413131) (IUI), *W.C. Lee 0022952* (KWNU), *W.C. Lee 0022953* (KWNU), *W.C. Lee et al. 064119* (KWNU), *W.C. Lee et al. 064146* (KWNU), *W.C. Lee et al. 066496* (KWNU), *Y.D. Kim 0505704-265* (KH), *Y.H. Cho WR-060603-014* (KH), *Y.H. Cho WR-071031-058* (KH), *Y.M Kim s.n.* (specimen no. 032146) (IUI).

Asparagus davuricus **Fisch. ex Link**

Korea: *B.H. Choi et al. s.n.* (specimen no. 033777) (IUI), *B.H. Choi et al. s.n.* (specimen no. 0037087) (IUI), *B.H. Choi et al. s.n.* (specimen no. 0037486) (IUI), *B.H. Choi s.n.* (specimen no. 033607) (IUI), *C.H. Shin s.n.* (specimen no. 16348) (IUI), *Choi et al. 609* (IUI), *H.B. Shim s.n.* (specimen no. 034890) (IUI), *H.J. Choi s.n.* (specimen no. 12690) (IUI), *J.H. Lee 73063* (IUI), *J.H. Lee 73065* (IUI), *J.K. Lee s.n.* (specimen no. 017882) (IUI), *M.K. Choi s.n.* (specimen no. 16369) (IUI), *S.H. Cho & Y.I. Kim ChoSH-006* (HHU), *S.H. Cho & Y.I. Kim ChoSH-007* (HHU), *S.H. Cho & Y.I. Kim ChoSH-008* (HHU), *S.H. Cho & Y.I. Kim ChoSH-009* (HHU), *S.H. Cho & Y.I. Kim ChoSH-010* (HHU), *S.H. Cho et al. ChoSH-020* (HHU), *S.H. Cho et al. ChoSH-025* (HHU), *S.H. Cho et al. ChoSH-027* (HHU), *S.H. Park p0112* (KH), *S.H. Park p0263* (KH), *S.Y. Yoo et al. s.n.* (specimen no. 16346) (IUI), *S.Y. Yoo et al. s.n.* (specimen no. 16357) (IUI), *W.C. Lee 0022933* (KWNU), *W.C. Lee 0022934* (KWNU), *W.C. Lee 0022935* (KWNU), *W.C. Lee 0022936* (KWNU), *W.C. Lee 0022937* (KWNU), *W.C. Lee 0022938* (KWNU), *W.C. Lee 0022942* (KWNU), *W.C. Lee 0022943* (KWNU), *Y.S Seok s.n.* (specimen no. 029909) (IUI).