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Species-Level Diversity in the Botanical Origin of Asparagi Radix (*Asparagus cochinchinensis* and its Allied Species: *Asparagaceae*) Distributed in China and Japan Revealed by DNA Barcoding

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To clarify species-level diversity in the botanical origin of Asparagi radix distributed in markets in China and Japan, we developed an identification method using DNA barcoding markers for *Asparagus cochinchinensis* and its allied species, and performed market investigations. Nucleotide sequences of the ITS regions were determined in 107 accessions of 21 *Asparagus* species, along with partial *trnL-trnF* intergenic spacer regions from a limited number of species. The examined species were discriminated from each other, except *A. meioclados* and *A. trichoclados*. Among 238 accessions of Asparagi radix distributed in markets, the most common origin was *A. cochinchinensis* (56%), followed by *A. subscandens* (25%), *A. taliensis* (16%), *A. lycopodineus* (2%), and *A. meioclados* or *A. trichoclados* (0.4%). All species were found in Chinese markets, whereas only three species, *A. cochinchinensis*, *A. taliensis*, and *A. subscandens*, were found in Japanese market. While four species were found to consist of only wild origin, *A. cochinchinensis* and *A. taliensis* were found to have both wild and cultivated origins. More than half of the *A. subscandens*-derived crude drugs

in the Chinese markets was estimated to be sourced from Myanmar based on the intraspecific barcoding variation, even though 55 out of 60 samples were sold as products originating from China.

Key words: *Asparagus cochinchinensis*, Asparagi radix, *Asparagus subscandens*, *Asparagus taliensis*, botanical origin, crude drug, DNA barcoding, market survey.

Asparagi radix is defined as the swollen tuberous root of *Asparagus cochinchinensis* (Lour.) Merr. (*Asparagaceae*), which is boiled or steamed, and from which most of the outer cortex and cork layer is removed (Namba 1993). This crude drug appeared in "Shennong Ben Cao Jing"（神農本草経）[Shen Nong's Herbal Classic Scripture], one of the oldest and most important classic books of the oriental medicine, written between about 206 B.C. and 220 A.D. It has been used as cough remedy, diuretic, symptom relief, for nourishing, and as a tonic（鎮咳，利尿，緩和，滋養，強壮）in Kampo and Chinese traditional medicine (Namba 1993, Xiao 2002).

Asparagi radix is prescribed as "Tenmondo（テンモンドウ）" in the Japanese Pharmacopoeia Eighteenth Edition (Ministry of Health Labour and Welfare of Japan 2021) and as "Tiandong（天冬）" in Pharmacopoeia of the People's Republic of China 2020th edition (Chinese Pharmacopoeia Commission 2020). Both pharmacopoeias limit the botanical origin of Asparagi radix to a single species, *Asparagus cochinchinensis*. As a crude drug similar to Asparagi radix, "XiaoTiandong（小天冬）" is prescribed in "Sichuan-sheng Zhongyaocai Biaozhu"（四川省中药材标准）[Standard of Chinese Herbal Medicines in Sichuan Province] 2010th edition, in which *A. meioclados* H.Lév. is designated as the botanical origin of it, and is produced in Guizhou, Sichuan, and Yunnan of China (Xie 2019).

However, regarding Asparagi radix produced in Sichuan, Zhang and Qin (1992a) reported the following: products originated from *Asparagus meioclados* increased from 1973; those from *A. filicinus* Buch.-Ham. ex D.Don were mixed; those from *A. lycopodineus* (Baker) F.T.Wang & Tang were used for private use; those from *A. myriacanthus* F.T.Wang & S.C.Chen were used in the 1960s but were not used after that; those from *A. kansuensis* F.T.Wang & Tang ex S.C.Chen were used as a substitute in some areas. Xiao (2002) reported the use of *A. myriacanthus* in Xizang, *A. subscandens* F.T.Wang & S.C.Chen in southern Yunnan, and *A. filicinus* and *A. lycopodineus* in a comparatively wide range. Hao et al. (2011) reported that *A. acicularis* F.T.Wang & S.C.Chen was also used as Asparagi radix. Su et al. (2013) reported that Asparagi radix originated from *A. taliensis* F.T.Wang & Tang ex S.C.Chen and *A. subscandens* were distributed in addition to *A. cochinchinensis* in Yunnan. Lu et al. (2019) reported that *A. meioclados*, *A. munitus* F.T.Wang & S.C.Chen, and *A. filicinus* were used in addition to *A. cochinchinensis*.

The advancement of cultivation of Asparagi radix may affect the diversity of its original species. The decline in the wild resources of Asparagi radix in recent years has been advocated by various articles, with Lu (2009) being one example. In response to this, the cultivation of Asparagi radix has been expanding. Shifting to cultivation is generally expected to reduce the occurrence of mixing with different species because cultivated products can cover a larger quantity and have lower prices. However, in the case of Asparagi radix, the shift to cultivation may contribute to increase mixing with different species. Lu et al. (2019) mentioned that the cultivated strains of such species as *Asparagus subscandens*,

A. munitus, *A. lycopodineus* and so on, were mixed with the main cultivars that originated from *A. munitus*. Su et al. (2013) showed cultivated *Asparagus* plants in photographs, but these plants did not appear to be *A. cochinchinensis* but rather *A. taliensis* based on the morphological criteria indicated by Chen and Tamanian (2000). During the process of collecting samples in our study, many of the commercially cultivated populations were morphologically identified as *A. cochinchinensis* or *A. taliensis*. *Asparagus munitus* was not found in cultivated samples but in wild ones. It is possible that the report of *A. munitus* in cultivation (e.g., Lu et al. 2019) may have been confused with *A. taliensis*.

In order to ensure the consistent quality of crude drugs, it is important to identify the original plant species that are actually being distributed in the markets. Recently, DNA barcoding markers such as chloroplast DNA (cpDNA) and the internal transcribed spacer regions of nuclear ribosomal DNA (the ITS regions) have been employed for detecting the origin of crude drugs such as Baizhu and Cangzhu (*Atractylodes* spp.; Mizukami et al. 2000, Shiba et al. 2006), Ginseng (*Panax* spp.; Zhu et al. 2003), and Xingren (*Prunus* sect. *Armeniaca*; Yamaji et al. 2009). Given that several precedent studies reported intra- and interspecific variation in the ITS regions and microsatellite loci for *Asparagus cochinchinensis* and the genus *Asparagus* (Fukuda et al. 2011, Ou et al. 2011, Lee et al. 2019), the ITS regions will be useful for investigating species composition in Asparagi radix distributed in China and Japan. However, there is little molecular information available for species allied to *A. cochinchinensis* mentioned above, which have tuberous roots.

In our preliminary investigation, it became apparent that *Asparagus taliensis* and *A. munitus* were similar in their ITS sequences. Therefore, a thorough investigation was specifically conducted to examine the presence of cultivated *A. munitus* in the market, with a particular focus on distinguishing the two species.

In the present study, we aimed to (1) develop DNA barcoding markers for *Asparagus* species, especially those potentially used for Asparagi radix, based on original plant samples of Asparagi radices and their allied species, and (2) to elucidate species-level diversity of Asparagi radix distributed in Chinese and Japanese markets.

Materials and Methods

Plant samples

In this study, 107 accessions were collected including cultivated and wild samples Asparagi radix obtained from our own field surveys. All samples were identified using morphological criteria followed by Chen (1978a, b) and Chen and Tamanian (2000). The voucher specimens of collected samples in this study were deposited in the herbarium of Tsumura Laboratory (THS). Their sources and voucher information are listed in Appendix 1.

During the investigation of the production areas, we collected samples of *Asparagus cochinchinensis* and *A. taliensis*, actually used as Asparagi radix. Second, we collected related species mentioned in the precedent studies (e.g., Xie 2019) for DNA analysis. According to Chen and Tamanian (2000), 12 of 31 species in the genus *Asparagus* distributed in China have tuberous roots. We examined nine species among them [*A. cochinchinensis*, *A. taliensis*, *A. meioclados*, *A. filicinus*, *A. munitus*, *A. lycopodineus*, *A. subscandens*, *A. densiflorus* (Kunth) Jessop., *A. racemosus* Willd.] and four species for which the presence of tuberous root was not explicitly stated [*A. officinalis* L., *A. setaceus* (Kunth) Jessop, *A. tibeticus* F.T.Wang & S.C.Chen, and *A. trichoclados* (F.T.Wang & Tang) F.T.Wang & S.C.Chen]. We collected living material from their natural habitats or botanical gardens as far as possible. However, the samples that

were particularly challenging to collect in their natural habitats (cf. *A. myriacanthus*) were obtained from herbarium specimens deposited in Kunming Institute of Botany, Chinese Academy of Sciences (KUN), Tsumura Laboratory (THS), and National Museum of Nature and Science, Japan (TNS).

In addition, the sequences of other *Asparagus* species and outgroup species from genera closely related to *Asparagus* (refer to Fukuda et al. 2005), were obtained from GenBank (Appendix 1).

Crude drug samples

Public crude drug market surveys in China were conducted in Xinluosi（新螺蛳）in 2014, Hehuachi（荷花池） in 2014 and 2019, Anguo（安国）, Bozhou（亳州）, and Yulin（玉林） from 2019 to 2020, and a total of 64 samples were obtained. For market survey in Japan, 33 crude drug samples were obtained from pharmaceutical companies between 2010 and 2020 (Appendix 2).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from a few dried cladodes on herbarium specimens and fragments from each tuberous root on crude drug samples, using DNeasy® Plant Mini Kit (QIAGEN) or QIAamp® DNA Stool Kit (QIAGEN). For each crude drug sample, 1 to 7 roots were evaluated.

Firstly, for all samples, the sequences of the ITS regions (the ITS1 spacer, 5.8S rRNA gene, and the ITS2 spacer) were determined. For some relatively old herbarium specimens and most of the crude drug samples, PCR amplification of the entire ITS regions using the universal primers presented by White et al. (1990) was not successful. Therefore, PCR amplification for all herbarium specimens was conducted in a two-stage trial. In the first stage, the ITS1 spacer and the ITS2 spacer were separately amplified using the universal primer pairs of “ITS5” & “ITS2” and “ITS3” & “ITS4”, respectively. In the second stage, based on the sequences detected in the first stage, some internal forward and reverse primers were newly designed (Table 1). By combining these primers, multiple short parts of the ITS regions were amplified. For crude drug samples, we used the second stage primers used for herbarium specimens from the beginning. As a part of the crude drugs distributed in the market are possibly derived from *Asparagus* species not identified in this study, we also conducted investigations of the entire length of the ITS regions in crude drug samples as far as possible. This allowed us to evaluate whether species other than those identified in this study were being distributed or not.

In order to discriminate *Asparagus taliensis* and *Asparagus munitus* in the form of crude drugs clearly, we developed a method using cpDNA sequences. Based on the screening of 15 cpDNA regions (*matK*, *ndhC-trnV*, *rbcL-accD*, *rps16-trnQ*, *rpl32-trnL*, *ndhF-rpl32*, *psbE-petL*, *trnT-psbD*, *petA-psbJ*, *trnT-trnL*, *trnL* intron, *trnL-trnF*, *petD-rpoA*, *rpl16* intron, *psbA-trnH*), we identified *trnL-trnF* intergenic spacer (Taberlet et al. 1991) as an useful maker to discriminate the two species. For herbarium specimens, we employed the universal primer pair of “e” & “f”. For crude drug samples identified as *A. taliensis* or *A. munitus* based on the ITS sequences, partial sequence of *trnL-trnF* intergenic spacer was detected using newly designed primer “4F” with the universal primer “f”, which reduced amplified length from 242 bp to 166 bp covering all variaton sites found in herbarium specimens. Primer pairs for amplification are given in Table 1.

The PCR amplification was performed in 28 μL of the reaction mixture, two type compositions were used properly corresponding to primer pairs; Type 1 mixture [Gene *Taq* (5 unit/μL; Nippon Gene, Japan) 0.14 μL, 10×Gene *Taq* Buffer 2.8 μL, dNTP mix (each 2.5 mM/L) 2.2 μL, DMSO 2.8 μL,

forward primer (10 pM/μL) 1.0 μL, reverse primer (10 pM/μL) 1.0 μL, template DNA 1 μL (the concentration was undetermined), and D.D.W. (deionized distilled water) 17.02 μL] and Type 2 mixture [*Ex Taq* (5 unit/μL; TaKaRa Bio, Japan) 0.14 μL, 10×*Ex Taq* Buffer 2.8 μL, dNTP mix (each 2.5 mM/L) 2.2 μL, forward primer (10 pM/μL) 1.0 μL, reverse primer (10 pM/μL) 1.0 μL, template DNA 1 μL (the concentration was undetermined), and D.D.W. 19.82 μL].

PCR reactions were performed using four kinds of thermal cycling conditions corresponding to primer pairs shown in Table 1, using Veriti® Thermal Cycler (Thermo Fisher Scientific, USA) or TProfessional Basic Thermal Cycler (Biometra Ltd., UK); Cycle 1 [94 °C, 4 min, (95 °C, 30 s; 70 °C, 15 s; 72 °C, 15 s) × 3 cycles, (95 °C, 30 s; 66 °C, 15 s; 72 °C, 15 s) × 3 cycles, (95 °C, 30 s; 62 °C, 15 s; 72 °C, 15 s) × 3 cycles, (95 °C, 30 s; 58 °C, 15 s; 72 °C, 15 s) × 3 cycles, (95 °C, 30 s; 54 °C, 15 s; 72 °C, 15 s) × 3 cycles, (95 °C, 30 s; 48 °C, 1.5 min; 72 °C, 2.5 min) × 20 cycles, 72 °C, 7 min], Cycle 2 [95 °C, 5 min, (95 °C, 30 s; 48 °C, 30 s; 72 °C, 1 min) × 35 cycles, 72 °C, 7 min], Cycle 3 [95 °C, 5 min, (95 °C, 30 s; 55 °C, 30 s; 72 °C, 1 min) × 35 cycles, 72 °C, 7 min], and Cycle 4 [95 °C, 5 min, (95 °C, 30 s; 55 °C, 30 s; 72 °C, 1 min) × 40 cycles, 72 °C, 7 min.]

PCR products were purified in two ways: (1) separating from other byproducts using 2% agarose gel electrophoresis, cutting down desired bands, and purifying by Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, USA), and (2) purifying directly by ExoSAP-IT™ Express (Thermo Fisher Scientific, USA). The purified PCR products were reacted using BigDye™ Terminator Cycle Sequencing Kit ver. 3.1 (Thermo Fisher Scientific, USA) with the same primer pairs used in PCR amplification. The thermal cycling condition was 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. After removal of unincorporated fluorescent reagents by ethanol precipitation, PCR products were sequenced in both directions using 3500 Genetic Analyzer (Thermo Fisher Scientific, USA). The obtained sequences were edited and aligned using the Contig Express program, a component of Vector NTI Advance (Version 9.1.0, Thermo Fisher Scientific, USA), CodonCode Aligner (Version 9.0.1, CodonCode Corporation, Dedham, MA, USA) and the BioEdit software (version 7.0.5.3, http://www.mbio.ncsu.edu/BioEdit/bioedit.html). A final consensus sequence was obtained by assembling the overlapped sequences in both directions.

Phylogenetic analysis

Phylogenetic relationships were reconstructed based on the maximum likelihood (ML) method using MEGA X (Kumar et al. 2018). "Find Best DNA/Protein Models" program was applied, and Kimura 2-parameter model was employed. The confidence of branching was assessed with 1000 bootstrap replicates. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5709, calculated in this dataset)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Results

Nucleotide sequences of the ITS regions

The sequences of the ITS regions were obtained from a total of 345 samples, including 107 herbarium specimens and 238 crude drug samples. Sixteen sequences, including four sequences of outgroups, were available from

GenBank (Appendix 1, 2). For the herbarium specimens, 78 samples were successfully determined in all regions which could be read by outermost primer pairs ("ITS5" & "ITS4"). For crude drug samples, 20 samples were successfully determined in all the regions. The sequences of 14 *Asparagus* species obtained in this study and the GenBank-derived sequences adopted were 257–247 bp in length for the ITS1 spacer and 241–247 bp for the ITS2 spacer, which were not included seven *Asparagus* species adopted as outgroups: *A. aethiopicus* L., *A. asparagoides* (L.) Druce, *A. falcatus* L., *A. gonoclados* Baker, *A. retrofractus* L., *A. schoberioides* Kunth, and *A. trichophyllus* Bunge.

In cases where assembly was not successful, individual fragment sequences were registered separately. All sequences were deposited in the DDBJ/EMBL/GenBank database under the accession numbers listed in Appendices 1 and 2.

Phylogenetic analysis of Asparagus *species and Asparagi radix*

In order to apply as many mutation sites and samples as possible into the phylogenetic analysis, 240 sites of the ITS1 spacer, 133 sites of 5.8S rRNA gene, 250 sites of the ITS2 spacer, two sites of 28S rRNA gene, including insertions/deletions, were included in the matrix. Ambiguous base sites, such as R (A or G), Y (C or T), etc., were excluded from the analysis. Firstly 188 samples were used to simplify the phylogenetic tree. Subsequently, after integrating samples without differences in tree length, 91 samples were selected for a re-analysis.

In the ML analysis, only one best log-likelihood phylogenetic tree was obtained (log likelihood of tree is -3107.32; Fig. 1). The ML phylogenetic tree showed the following salient features: (1) *Asparagus cochinchinensis* was monophyletic, though the bootstrap values (BP) was as low as 62%, (2) *A. cochinchinensis* was divided into three subclades with high BP, AC1 (85%), AC2 (93%), and AC4 (98%), and one branch (AC3); among these, AC1 and AC2 were monophyletic (BP 99%), (3) *A. taliensis* and *A. munitus* formed a clade (BP 81%) but *A. munitus* was nested in *A. taliensis*, (4) the clade of *A. taliensis* and *A. munitus* was closely related to the clade of *A. cochinchinensis* (BP 98%), (5) each of *A. subscandens* and *A. lycopodineus* formed a distinct clade with high BP (92% and 90%, respectively), (7) *A. meioclados* and *A. trichoclados* formed a clade together with low BP (54%), but they could not be entirely differentiated, (8) *A. subscandens*, *A. lycopodineus*, *A. meioclados*, and *A. trichoclados* were monophyletic with moderate BP (69%), and (7) crude drug samples on the markets were placed in *A. cochinchinensis*, *A. taliensis* + *A. munitus*, *A. lycopodineus*, and *A. subscandens* clades.

Species discrimination sites and identification of crude drug samples

Due to the inability to fully detect the sequences of the ITS regions in many crude drug samples, species identification based on their phylogenetic positions was not feasible. Therefore, we developed a method for species identification based on the characteristics at specific sites of sequences of the ITS regions. Based on the phylogenetic relationship and variation observed in all the ITS sequences of herbarium specimens, we identified specific variation sites that were effective for species identification. A total of eighteen sites were found to be effective for species identification (Fig. 2), and these sites were categorized into three groups as follows (Figs. 1, 3).

Group 1 sites can distinguish each of the five major species group (*Asparagus cochinchinensis*, *A. taliensis* + *A. munitus*, *A. lycopodineus*, *A. subscandens*, *A. meioclados* + *A. trichoclados*) from other allied species (Figs. 1, 3). For instance,

Table 1. Primer sequences and primer pairs with their sequencing coverage and PCR condition. Primers used in the preliminary survey for chloroplast genomes are omitted.

Region / Primer name	Sequence (5' → 3')	Ref.*
ITS		
ITS5	GAAGTAAAAGTCGTAACAAGG	1)
ITS2	GCTGCGTTCTTCATCGATGC	1)
ITS3	GCATCGATGAAGAACGCAGC	1)
ITS4	TCCTCCGCTTATTGATATGC	1)
Asp-1F	CTGGGCGTCATGCCTCACATC	2)
Asp-1R	CCGGCCAGCGACCATCATTTC	2)
AspITS1-1F	GATCATTGTCGAGACCCGAGC	2)
AspITS1-1R	GTATTGTTCCCGCCKCCCGTTC	2)
AspITS1-2F	CTCCTYGGGCACCTATGGC	2)
AspITS1-2R	GCGATGCAGCACACGGYG	2)
AspITS1-3F	CGCCAAGGACTAGTGCTTTAG	2)
AspITS2-1R	CGATGTGAGGCATGACGC	2)
AspITS2-1F	GCTATYTGGCCGAGGGCAC	2)
AspITS2-2R	GACMATCATTTCAACCCRCCG	2)
AspITS2-2F	GAKATTGACCYCCCGTGCCTTG	2)
AspITS2-3R	CGCCGCWTTGGCTWAGGG	2)
AspITS2-3F	GACGCTGARCGYTGTGTAC	2)
AspITS2-4F	ACAYGRCGAATGGTGGAC	2)
AsMu1-4F	CCTYGGGCACCTATGGCCG	2)
AsMu1-4R	CGCTTAGCGATGCAGCACAC	2)
AsMu1-5F	GTCCTGCCTCGCWGCAGAAC	2)
AsMu1-5R	GGTATGATTACCGGACCACATCG	2)
AsMu1-6F	GAACAATACCCGGCGCGGTG	2)
AsMu1-6R	CCAAGATATCCGTTGCCAAGAGTC	2)
AsMu2-1F	CGCAGCGAAATGCGATACTTG	2)
AsMu2-1R	GCACGGAGCGATGTGAGG	2)
AsMu2-2F	CCGTGAACCATCGAGTCTTTGAAC	2)
AsMu2-2R	CGCCGCCGCTTCCCAATG	2)
AsMu2-3F	GCGTCATGCCTCACATCG	2)
AsMu2-3R	CCATTCGYCRTGTCCCACC	2)
AsMu2-4F	GCGCGGATGCGGAGATTG	2)
AsMu2-4R	CGCTTTGGCTTAGGGTCG	2)
AsMu2-5F	CGGYGGGTTGAAATGATKGTC	2)
AsMu2-5R	GCTCTTAAGCGCCCGTCG	2)
trnL-trnF intergenic		
e	GGTTCAAGTCCCTCTATCCC	3)
f	ATTTGAACTGGTGACACGAG	3)
4F	TGAAGATCTAAGAAATCGGG	2)

*References:
1) White et al. 1990, 2) this study, 3) Taberlet et al. 1991.

Region / Purpose	Primer pair (forward & reverse)	Sequencing coverage	Composition	Cycle
ITS				
Applied for herbarium specimens				
	ITS5 & ITS4	1–738	Type 1	1 or 2
	ITS5 & ITS2	1–321	Type 1	1 or 2
	ITS3 & ITS4	342–738	Type 1	1 or 2
Applied for crude drugs and old herbarium specimens				
	Asp-1F & Asp-1R	460–556	Type 1	1
	ITS5 & AspITS1-1R	1–159	Type 1	1
	AspITS1-1F & AspITS1-1R	46–159	Type 1	1
	AspITS1-2F & AspITS1-2R	137–223	Type 2	3 or 4
	AspITS1-3F & ITS2	219–321	Type 2	3 or 4
	ITS3 & AspITS2-1R	342–442	Type 2	3 or 4
	AspITS2-1F & AspITS2-2R	433–547	Type 2	3 or 4
	AspITS2-2F & AspITS2-3R	543–634	Type 2	3 or 4
	AspITS2-3F & ITS4	631–738	Type 2	3 or 4
	AspITS2-4F & ITS4	602–738	Type 2	3 or 4
	AsMu1-1F & AsMu1-1R	20–116	Type 2	3 or 4
	AsMu1-2F & AsMu1-2R	74–172	Type 2	3 or 4
	AsMu1-3F & AsMu1-3R	110–206	Type 2	3 or 4
	AsMu1-4F & AsMu1-4R	139–227	Type 2	3 or 4
	AsMu1-5F & AsMu1-5R	164–257	Type 2	3 or 4
	AsMu1-6F & AsMu1-6R	193–292	Type 2	3 or 4
	AsMu2-1F & AsMu2-1R	357–450	Type 2	3 or 4
	AsMu2-2F & AsMu2-2R	398–493	Type 2	3 or 4
	AsMu2-3F & AsMu2-3R	461–576	Type 2	3 or 4
	AsMu2-4F & AsMu2-4R	528–631	Type 2	3 or 4
	AsMu2-5F & AsMu2-5R	569–670	Type 2	3 or 4
trnL-trnF intergenic				
Applied for herbarium specimens				
	e & f	1–201	Type 1	3
Applied for crude drugs				
	4F & f	76–201	Type 2	4

A. cochinchinensis has either "A, G, or R" at site 580, while other species have "C." To distinguish *A. taliensis* and *A. munitus* from other *Asparagus* species, using site 486 was effective. Similarly, *A. lycopodineus* can be distinguished by using sites 390 and 695, *A. subscandens* by using sites 146, 424, and 463, and *A. meioclados* and *A. trichoclados* can be distinguished from other *Asparagus* species by using site 156 (Fig. 3).

Group 2 sites were shared in the clade of *Asparagus cochinchinensis*, *A. taliensis*, and *A. munitus*, as well as in the clade of *A. subscandens*, *A. lycopodineus*, *A. meioclados*, and *A. trichoclados*, but not in allied species (Figs. 1, 3). For example, at site 243, *A. cochinchinensis*, *A. taliensis*, and *A. munitus* have "A," while other species have

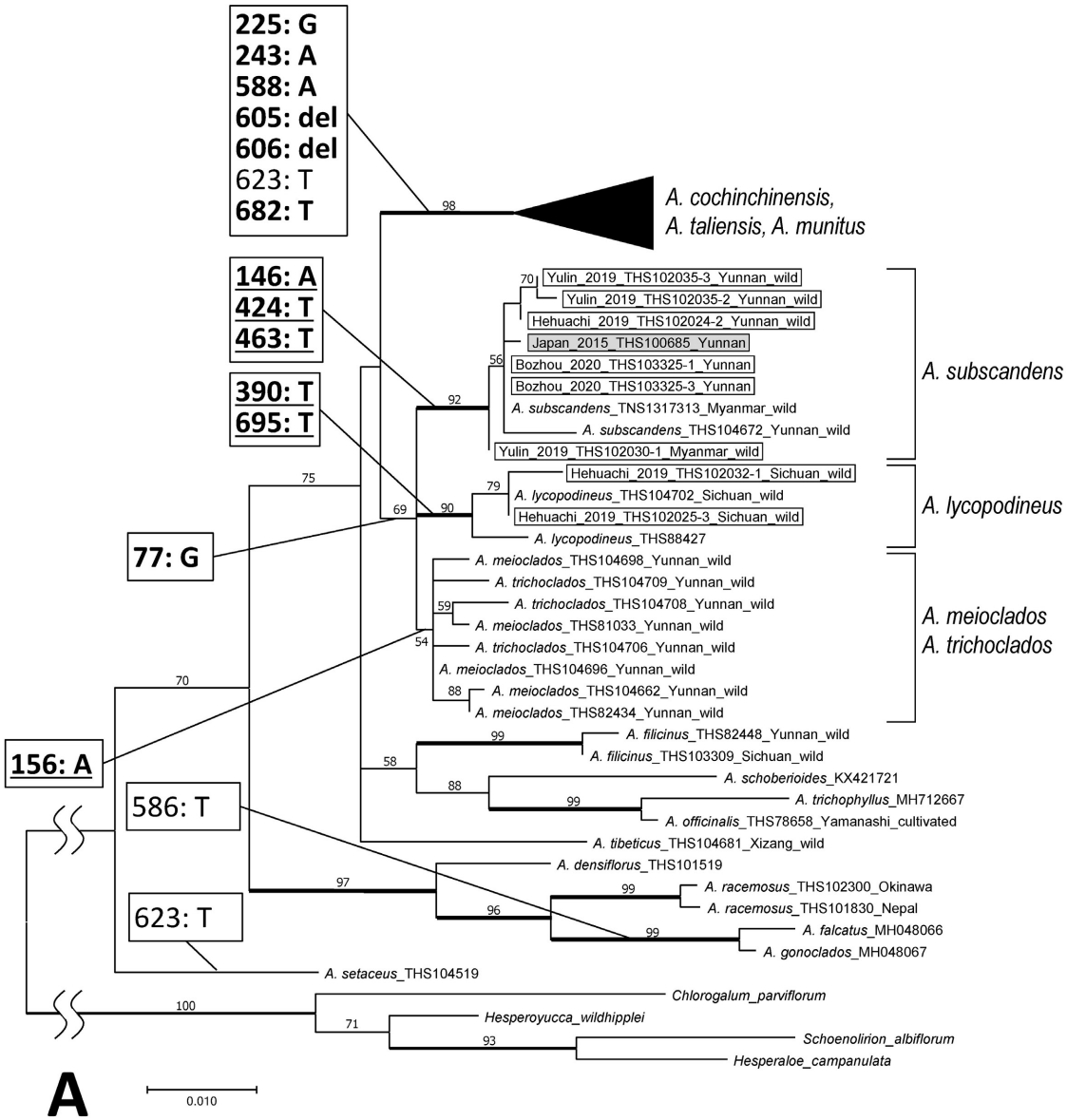

Fig. 1. The best log-likelihood ML phylogenetic tree for original plants and their allied species of Asparagi radix based on sequences of the ITS regions (ITS1 and ITS2 spacers, partial 5.8S, 18S, and 28S rRNA genes). A. Overall tree: the phylogenetic position of the clade indicated in B is marked at the top of the tree with a solid black triangle. B. Partial tree within the clade composed of *Asparagus cochinchinensis*, *A. taliensis*, and *A. munitus*. The log likelihood of the tree is -3107.32. Bootstrap values of branches (over 50%) are shown, with branches possessing high bootstrap values (over 90%) indicated by thick lines. Crude drug samples are framed in black lines, and samples from markets in Japan are shaded. The phylogenetic placement of character changes on species-discriminating sites (refer to Fig. 3) is shown on each branch. Three grouping of these sites were categorized as follows: Group 1 (bold, underlined), Group 2 (bold), Group 3 (regular). The distinction between Groups 1–3 was described in the main text.

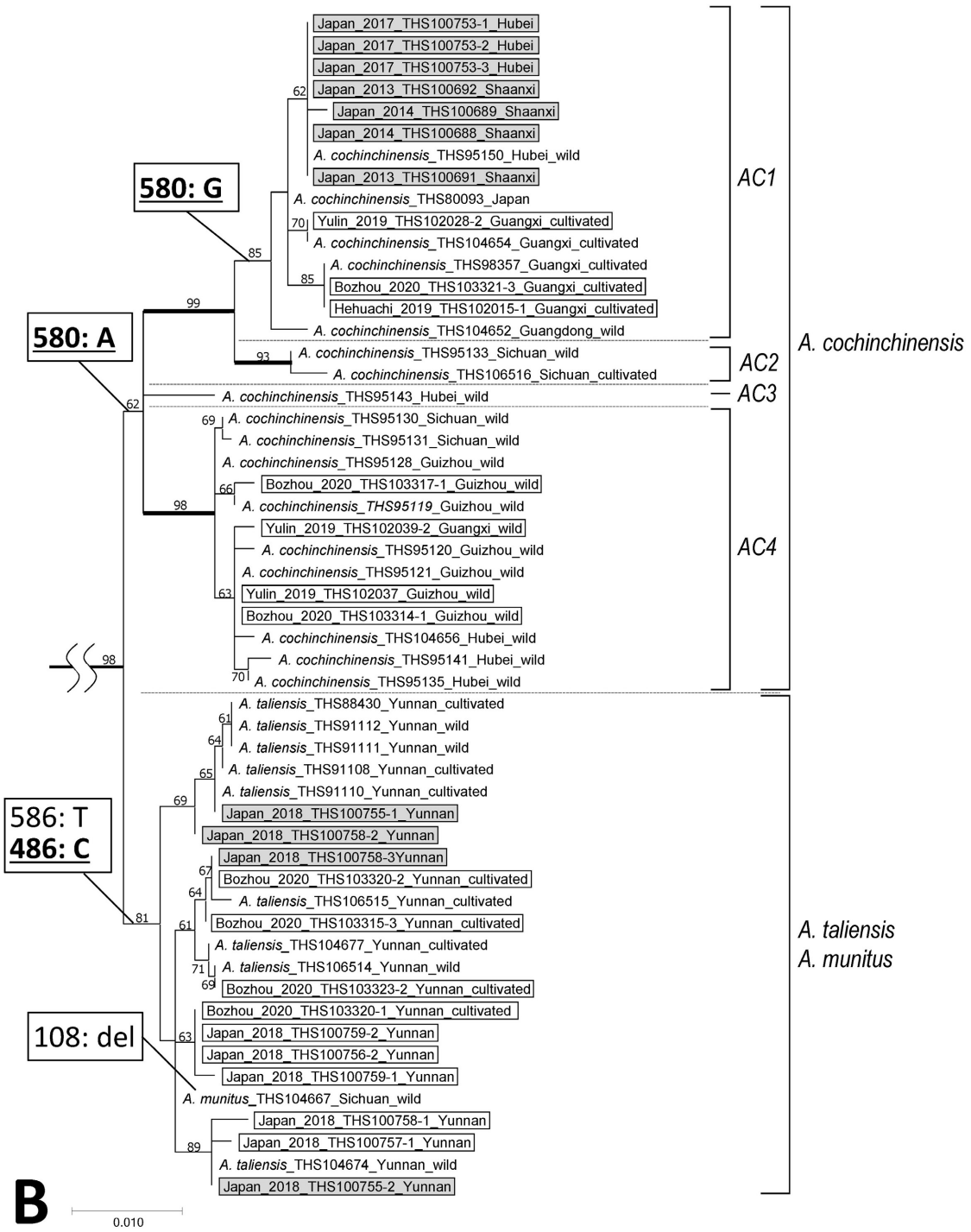

Fig. 1. Continued.

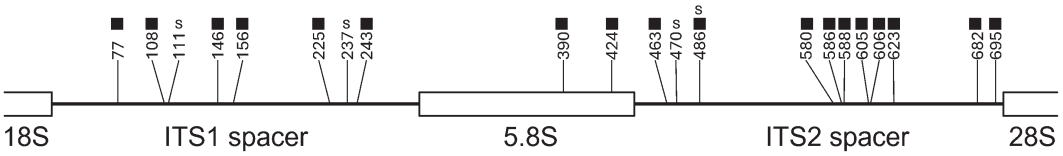

Fig. 2. The positions of variable sites of the ITS sequences, including 3' end of 18S rRNA gene and 5' end of 28S rRNA gene, detected among 14 *Asparagus* species and Asparagi radix samples examined in this study. Symbols and letters above numbers indicate as follows: black squares (■) are useful sites for species discrimination (refer to Fig. 3), "s" is intraspecific variation sites within *A. subscandens* (refer to Fig. 7).

"C, T or Y."

Group 3 sites were similar to Groups 1 and 2 but were also found in other *Asparagus* species (Figs. 1, 3). For instance, at site 623, *A. cochinchinensis*, *A. taliensis*, and *A. munitus* have "T," which is effective in distinguishing them from other major species, but one of the allied species, *A. setaceus*, also has "T."

The determination of the targeted sites refers to the range determined by each primer shown in Table 1, and the appropriate primers are selected accordingly. For example, site 580 of Group 1, as above mentioned, is included within the region amplified using primer pairs "ITS3 & ITS4" (Fig. 2, Table 1). When dealing with crude drug samples that may have DNA fragmentation, the primer pair of "AspITS2-2F & AspITS2-3R," which amplifies shorter fragments, is available. Nevertheless, in some crude drug samples, it was not possible to determine Group 1 sites. In such cases, we attempted to conduct species identification using Group 2 and Group 3 sites and the overall similarity of other sites. As a result, no crude drug samples occupied a unique phylogenetic position or had sequences distinct from the species examined in this study.

While *Asparagus munitus* and *A. taliensis* can be distinguished from other species by site 486 of Group 1, these two species were not differentiated (Fig. 3). However, a difference in the repeat number of "C" (poly-C) at site positions 108–112 was found between *A. munitus* (four) and *A. taliensis* (five). Nevertheless, its stability for species identification was questionable because the repeat number of a single nucleotide is known as evolutionarily unstable (Varshney et al. 2005, Vieira et al. 2016). On the other hand, in the *trnL-trnF* intergenic spacer, these two species examined with morphologically identified herbarium specimens were divided into four types by five nucleotide substitutions (Fig. 4): type 1 for *A. munitus* and types 2–4 for *A. taliensis*. Among the 39 crude drug samples of *A. taliensis* or *A. munitus* identified by ITS sequences, the sequences of *trnL-trnF* intergenic spacer in 32 samples successfully agreed with one of the four sequence types. The remaining three samples shared a single sequence type (type 5), which shares the unique allele G of *A. taliensis* in site 188. Subsequently as *A. taliensis* and *A. munitus* showed consistent difference within the samples examined in the repeat number of "C" (poly-C) at site positions 108–112 in the ITS regions and at in site 188 in *trnL-trnF*, these two species can be identified based on the ITS regions.

Trends in original plant species of crude drug samples and their geographical origins, markets, and type of production

In proportion to the original plant species of crude drug samples in the Chinese and Japanese markets revealed by DNA barcoding (Fig. 5A), the most common species in the markets was *Asparagus cochinchinensis* (133 samples, 56%), followed by *A. subscandens* (60 samples, 25%), *A. taliensis* (39 samples, 16%), *A. lycopodineus* (5 samples, 2%), and

Species \ Position	Group 1								Group 2							Group 3		
	580	486	390	695	146	424	463	156	77	225	243	588	605	606	682	108	586	623
Major Species																		
A. cochinchinensis	**A/G/R**	A/G/R	C	C	C	C	C	T	A	**G**	**A**	**A**	**d**	**d**	**T**	C/T/d*	C	**T**
A. taliensis	C	**C**	C	C	C	C	C	T	A	**G**	**A**	**A**	**d**	**d**	**T**	C	**T**	**T**
A. munitus	C	**C**	C	C	C	C	C	T	A	**G**	**A**	**A**	**d**	**d**	**T**	**d***	**T**	**T**
A. lycopodineus	C	G/T/K	**T**	**T**	C/Y	C	C	T	**G**	A	C	G	C	A	C	G	C	C
A. subscandens	C	G/T	C	C	**A**	**T**	**T**	T	**G**	A	C	G	C	A	C	G	C	C
A. meioclados, A. trichoclados	C	G	C	C	C	C	C	**A**	**G**	A	C	G	C	A	C/Y	G/T/K	C	C
Allied Species																		
A. filicinus	C	G	C	C	C	C	C	T	A	A/R	C	G	C	A	C	G	C	C
A. myriacanthus	?	T	?	?	?	?	C	?	?	?	?	?	?	?	?	?	?	?
A. densiflorus	C	G	C	C	C	C	C	T	A	A	C	G	C	T	C	G	C	C
A. setaceus	C	G	C	C	C	C	C	T	A	A	T	G	C	A	C	G	C	**T**
A. officinalis	C	G	C	C	C	C	C	T	A	A	C	G	C	A	C	A	C	C
A. racemosus	C	T	C	C	C	C	C	T	A	A	C	G	C	A	C	G	C	C
A. tibeticus	C	G	C	C	C	C	C	T	A	A	Y	G	T	A	C	G	C	C
A. asparagoides	C	G	C	C	C	C	C	T	A	A	T	G	T	A	C	T	C	C
A. falcatus	C	T	C	C	C	C	C	T	A	A	C	G	C	A	C	G	**T**	C
A. gonoclados	C	T	C	C	C	C	C	T	A	A	C	G	C	A	C	G	**T**	C
A. schoberioides	C	G	C	C	C	C	C	T	A	A	T	G	C	A	C	A	C	C
A. retrofractus	C	G	C	C	C	C	C	T	A	A	C	G	C	A	C	G	C	C
A. trichophyllus	C	G	C	C	C	C	C	T	A	A	C	G	C	A	C	A	C	C
A. aethiopicus	C	G	C	C	C	C	C	T	A	A	C	G	C	T	C	G	C	C

Fig. 3. Character states at species-discriminating sites of ITS sequence for the major seven *Asparagus* species and their allied species. The distinction between Groups 1–3 was described in the main text. Question mark (?) indicates that the nucleotide at each site in that species could not be determined. "Character state "d" indicates nucleotide deletion. Character state "d*" indicates C × 4 (refer to Fig. 4)."

A. meioclados or *A. trichoclados* (1 sample, 0.4%).

A comparison by source locality (Fig. 5A) reveals that only *Asparagus cochinchinensis* was found in crude drug samples from Hubei, Guangxi, Chongqing, and Shaanxi. However, in addition to *A. cochinchinensis*, *A. lycopodineus* was found in ones from Sichuan, while *A. taliensis* and *A. subscandens* were found in ones from Guizhou. Samples from Yunnan exhibited the largest species-level diversity; *A. cochinchinensis* only had a 7% share (16 out of a total of 106 individuals), and *A. taliensis*, *A. subscandens*,and *A. meioclados* or *A. trichoclados* was found. Only *A. subscandens* was found in samples from Myanmar.

On species composition in each market (Fig. 5B), all species were found in Chinese markets, whereas only three species were found in the Japanese market: *Asparagus cochinchinensis*, *A. taliensis*, and *A. subscandens*. Although the ratios of species varied by the markets, there was no remarkable difference in constituent, except for Xinluosi, where only *A. taliensis* and *A. meioclados* or *A. trichoclados* were found.

In terms of the type of production (wild or cultivated origin; Fig. 5C), while four species were found to consist of only wild origin, *Asparagus cochinchinensis* and *A. taliensis* were found to have both wild and cultivated

Species, sample type, number	Position	*trnL-trnF* 85	92	105	152	188	Type	ITS 108–112
A. munitus HS	3	A	T	T	A	A	type 1	C × 4
A. taliensis HS	13	A	T	G	A	G	type 2	C × 5
CDS	14	A	T	G	A	G		C × 5
A. taliensis HS	1	A	T	G	G	G	type 3	C × 5
CDS	12	A	T	G	G	G		C × 5
A. taliensis HS	1	C	T	T	G	G	type 4	C × 5
CDS	3	C	T	T	G	G		C × 5
CDS	3	A	G	T	G	G	type 5	C × 5

Fig. 4. Character states at variable sites on the sequences of *trnL-trnF* intergenic spacer and at site 108 of the ITS1 spacer for samples identified as *Asparagus taliensis* and *A. munitus*. Samples with the same status are grouped together, with the number of individuals indicated in "Species, sample type, number" column. In the sample type, "HS" and "CDS" indicate "herbarium specimen", "crude drug sample", respectively.

origins. In the case of *A. taliensis*, the number of cultivated ones was more than three times greater than that of the wild ones.

Characteristics of Asparagi radix of various origins

A comparison of the crude drug samples divided by their botanical origin showed the following salient features (Fig. 6): (1) samples originated from wild *Asparagus cochinchinensis* were generally dark in color, and the size and shape varied widely (Figs. 6A, B), (2) samples originated from cultivated *A. cochinchinensis* in Guangxi and Sichuan showed low variation, appearing pale yellow, and generally small and thin (Fig. 6C, D), (3) samples originated from cultivated *A. taliensis* in Yunnan showed low variation, appearing pale yellow, and comparatively large and well filled (Fig. 6E, F). This matches the characteristics of superior quality products designated in Huang et al. (2017), (4) samples originating from wild *A. subscandens* in Yunnan and Myanmar had delamination on the surface and the inside, with wrinkled and whitish surface, but orange-brown inside (Fig. 6G–I), (5) samples originated from *A. meioclados* or *A. trichoclados* were similar to those from *A. subscandens* (Fig. 6J), and (6) samples originating from *A. lycopodineus* were small and slender, varying from monochromatic to brown (Fig. 6K, L).

Discussion

DNA barcoding marker for Asparagus species and Asparagi radix

In this study, we developed DNA barcoding markers for the identification of *Asparagus* species and Asparagi radices. As a result, we found that, except for distinguishing between *A. meioclados* and *A. trichoclados*, the ITS regions alone were sufficient for species discrimination. In addition, we found that *A. taliensis* and *A. munitus* are also differentiated in the sequence of *trnL-trnF* intergenic spacer.

In Chen (1978a, b), *Asparagus meioclados* was distinguished by erect, densely cartilaginous-denticulate stems, whereas *A. trichoclados*, which was firstly described as a variety of *A. meioclados* (Chen 1978a, b), has climbing, smooth stems. However, our samples [THS104705–THS104709] have such characters mixed, i.e. climbing stems whose surface was not smooth but sparsely cartilaginous-denticulate, suggest that their taxonomic status is still disputable.

As no crude drug samples had unique sequences in the ITS regions remarkably different from any of the herbarium specimens identified in this study, we concluded that all crude drug samples were derived from one of the species identified in this study.

In the investigation of *trnL-trnF* for *Asparagus taliensis* and *A. munitus*, type 5 was only found in crude drugs. However, these were considered to be *A. taliensis* based on the ITS regions.

Species-level diversity in the botanical origin of Asparagi radix

This study revealed that the botanical origin of Asparagi radix is more diverse than that

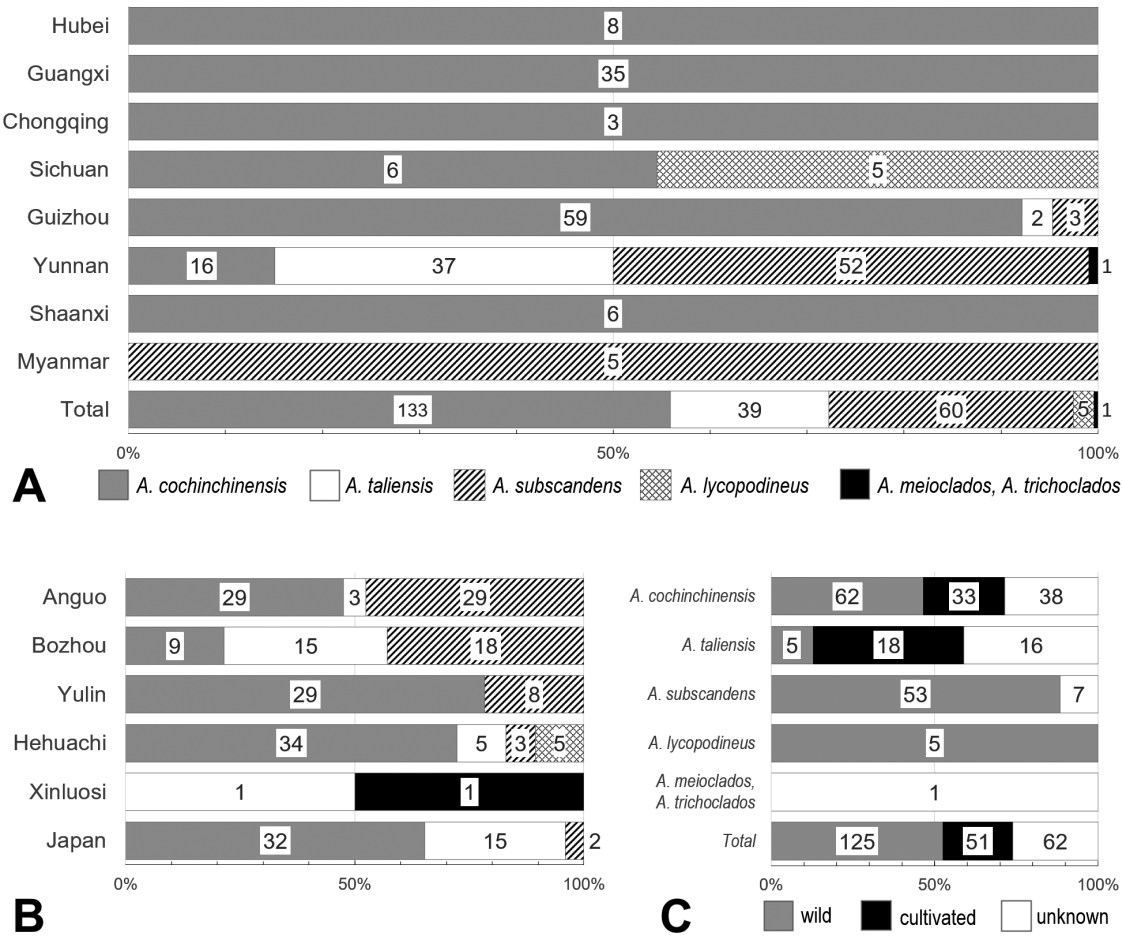

Fig. 5. Proportion and number of original plant species of Asparagi radix in each source locality (A), in each market (B) and proportion and number of wild, cultivated, and unknown products in each of the original plant species (C). The categorization through hatching is common to both A and B.

regulated in both the Japanese Pharmacopoeia Eighteenth Edition (2021) and Pharmacopoe ia of the People's Republic of China 2020 edition, which limits the botanical origin to *Asparagus cochinchinensis* only. In the Chinese markets, crude drugs distributed as Asparagi radix actually included those not only from *A. cochinchinensis* but also from *A. taliensis*, *A. lycopodineus*, *A. subscandens*, and *A. meioclados* or *A. trichoclados*. In the Japanese market, crude drugs from *A. taliensis* and *A. subscandens* were distributed together with those from *A. cochinchinensis*. The species other than *A. cochinchinensis* are discussed as follows.

1. *Asparagus taliensis* and *A. munitus*

In this study, no crude drug originated from *Asparagus munitus* was found. Based on herbarium specimen information in Chinese Virtual Herbarium (https://www.cvh.ac.cn/) and specimens in KUN, *Asparagus munitus* may not have been collected recently. Our surveys in the localities described in its original description (Chen 1978b) indicated that *A. munitus* was probably rare. Our interview survey around there revealed that it had been collected and used for medicine in the past and

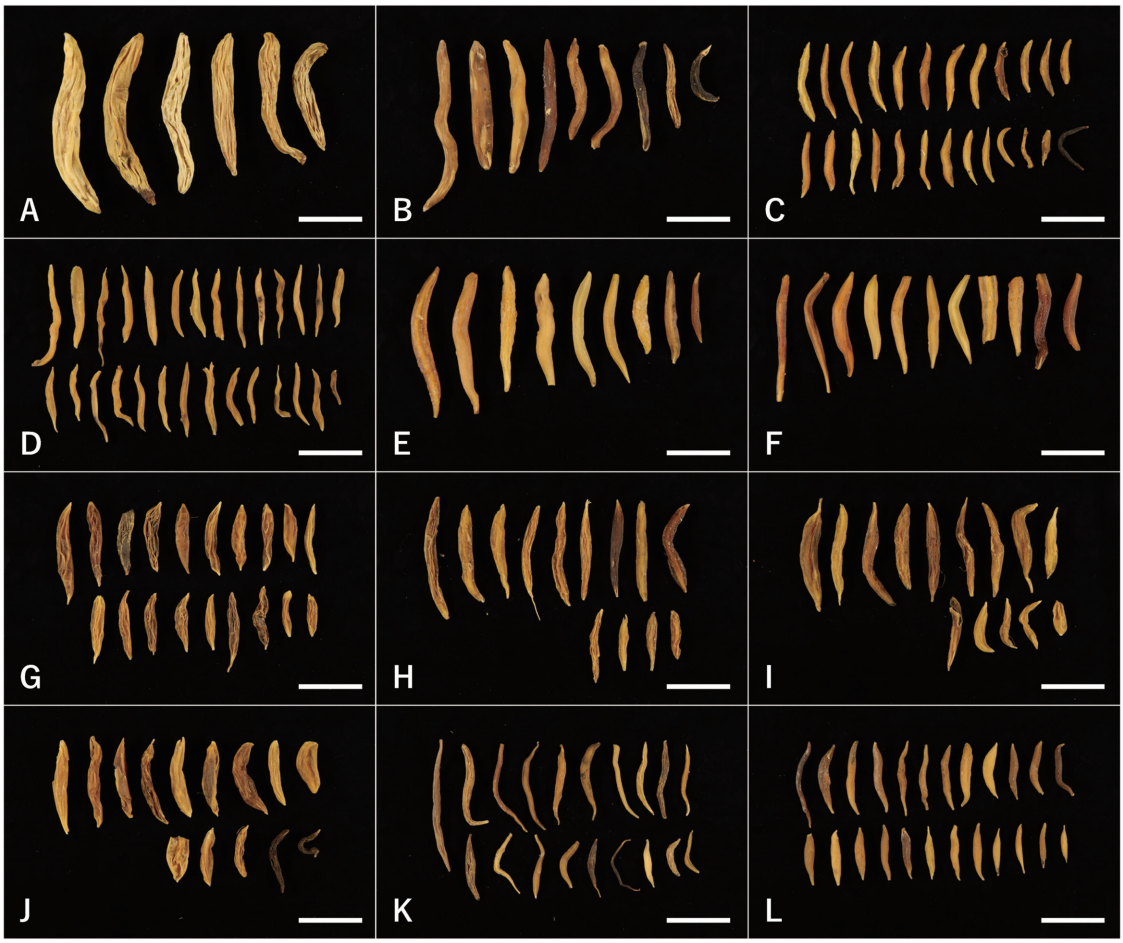

Fig. 6. Representative Asparagi radices from different species of *Asparagus*. A–D. *A. cochinchinensis* (A, B. Origin from wild harvested; C, D. Cultivated). A. Bozhou market, from Guizhou (THS103314). B. Yulin market, from Guangxi (THS102039). C. Yulin market, from Yulin-shi, Guangxi (THS102038). D. brought from Dr. Lu Xiang-yang, not used for DNA analysis, from Neijiang, Sichuan (THS106517). E, F. *A. taliensis* (cultivated). E. Bozhou market, from Lijiang-shi, Yunnan (THS103323). F. Bozhou market, from Yunnan (THS103326). G–I. *A. subscandens* (wild). Bozhou market, from Yunnan (G: THS103312, H: THS103313, I: THS103319). J. *A. meioclados* or *A. trichoclados*. Xinluosi market, from Dali-zhou, Yunnan (THS92088). K, L. *A. lycopodineus* (wild). K. Hehuachi market, from Leshan-shi or Yibin-shi, Sichuan (THS102025). L. Hehuachi market, from Yibin, Sichuan (THS102032). Scale bars = 5 cm.

had been taken almost all now. On the other hand, *A. taliensis* was cultivated practically in areas where cultivation of *A. munitus* was previously reported, suggesting that the two species may have been confused.

2. *Asparagus subscandens*

According to this study, we confirmed that a significant number of Asparagi radices originated from *Asparagus subscandens* were distributed in markets. Xiao (2002) had already reported the use of *A. subscandens* in southern Yunnan under various names such as Tianmendong（天门冬), Tutiandong（土天冬), Xiaojingye tiandong（小茎叶天冬), and Tubaibu（土百部).

We distinguished *Asparagus subscandens* from other allied species by following

characteristics: tuberous roots, erect or climbing stems without spines, and cladodes arranged in fascicles of 3–7, 3–6 × ca. 0.6 mm, falcate, flat or slightly 3-angled (Chen and Tamanian 2000). The species has been considered endemic to areas between 800 and 1700 m altitude in Southern Yunnan, China (Chen and Tamanian 2000), but we confirmed that the species is native to Myanmar, based on DNA barcoding as well as morphological features.

Among the herbarium specimens identified as *Asparagus subscandens*, six samples from Yunnan showed differences in five loci in comparison with one sample from Myanmar (M1 [TNS1317313]; Fig. 7). Another sample from Myanmar (M2 [TNS1329184]), although not fully determined for the entire ITS regions, shared four variations with M1, except for the undetected site 237. Thus, the Yunnan type (YN) and the Myanmar type (MM) of *A. subscandens* were distinguishable based on the five sites. Out of the 60 crude drug samples identified as *A. subscandens* (Fig. 5A), 29 samples were a much for all the five sites, while 19 samples muched four sites, except for site 237. Two samples matched the YN, 34 samples matched the MM, and 10 samples exhibited an intermediate state between the YN and MM. Therefore, it is suggested that more than half of the *A. subscandens*-derived crude drugs in the Chinese markets were sourced from Myanmar, even though 55 out of 60 samples were labeled and sold as products originating from China.

Samples	Locality	Det. count	Position 55	111	237	470	486	Type
HS (Y1–6)	Yunnan	6	C	A	G	C	G	YN
CDS	Yunnan	1	C	A	G	C	G	
	Yunnan	1	C	A	N	C	G	
CDS	Yunnan	1	Y	A	N	C	G	Intermediate
	Yunnan	1	Y	M	N	C	G	
	Yunnan	1	T	C	A	C	G	
	Yunnan	4	T	C	N	C	G	
	Yunnan	1	T	M	A	-	T	
	Yunnan	1	T	C	A	-	K	
	Guizhou	1	T	C	N	-	K	
HS (M1)	Myanmar	1	T	C	A	-	T	MM
HS (M2)	Myanmar	1	T	C	N	-	T	
CDS	Myanmar	4	T	C	A	-	T	
	Yunnan	20	T	C	A	-	T	
	Guizhou	2	T	C	N	-	T	
	Yunnan	8	T	C	N	-	T	

Fig. 7. Intraspecific variation of *Asparagus subscandens* in the ITS regions among herbarium specimens and crude drug samples identified as *A. subscandens*. Five sites were selected based on the differences between specimens from Yunnan (Y1~6: THS104671, 104672, 104685–104688) and ones from Myanmar (M1: TNS1317313, M2: TNS1329184). Only crude drug samples that could be detected at least four sites among these five sites are shown. Samples with the same status are grouped together, with the number of individuals indicated in the left column of the "Det. count" column. In the sample type, "HS" and "CDS" indicate "herbarium specimen", "crude drug sample", respectively. Based on similarity, they were classified into three types: Yunnan type (YN), Myanmar type (MM), and intermediate type between YN and MM. Additive states are represented by white-black inversion, states with complete base substitutions (appearing as such) are shaded, and undetected sites are indicated as "N".

3. *Asparagus lycopodineus*

Our result supported the report of Xiao (2002) on the use of *Asparagus lycopodineus* in Sichuan under the names of Tubaibu（土百部), Shanbaibu（山百部), Wuxiaotiandong（乌小天冬), and Wumaidong（乌麦冬).

4. *Asparagus meioclados* and *A. trichoclados*

In this study, one crude drug sample from Chinese market was identified as *Asparagus meioclados* or *A. trichoclados*. *Asparagus meioclados* was prescribed in Sichuan Chinese Materia Medica Standards 1987 as Tiandong（天冬), later the drug name was changed to "XiaoTiandong（小天冬)" in 2010 version of the same standard (Xie 2019).

Other Asparagus *species reported to be used as crude drugs*

In the past reports, in addition to the species mentioned above, *Asparagus filicinus*,

A. myriacanthus, *A. kansuensis*, and *A. officinalis* were reported to be mixed to Asparagi radix or used as a folk medicine (Zhang and Qin 1992b, Xiao 2002, Hao et al. 2011, Su et al. 2013, Lu et al. 2019, Xie 2019). However, in this study, crude drugs originated from these species were not found in markets.

Asparagus filicinus may not be treated as Asparagi radix due to its remarkably small size though this species is widely distributed in China (Chen and Tamanian 2000). Our interview survey revealed that the root of *A. filicinus* was locally used as a substitute for Stemonae radix（百部） or Ophiopogonis radix（麦門冬）. According to Xiao (2002), *A. filicinus* is distributed under the name of Tubaibu（土百部）. Though *A. officinalis* is widely cultivated as a vegetable, it may not be used as Asparagi radix because it lacks tubers. The reason why *A. myriacanthus* and *A. kansuensis* were not found in the markets was unknown.

By the use of DNA barcoding, this study revealed the existence of four to five species of *Asparagus* which are actually distributed as Asparagi radix, in addition to *A. cochinchinensis* which is exclusively prescribed in pharmacopoeias of Japan and China as source plants for Asparagi radix.

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References

Chen X.Q. 1978a. *Asparagus*. *In*: Wang F.Z., Tang J., Chen X.Q, Zhang Z.Y., Dai L.K., Liang S.Y., Tang Y.C., Liu L. and Lang K.Y. (eds.), Fl. Reipubl. Popularis Sin. **15**: 98–122. Science Press, Beijing (in Chinese).

Chen X.Q. 1978b. Species novae generis Asparagi e flora Sinica. Acta Phytotax. Sin. **16**: 91–95 (in Chinese).

Chen X.Q. and Tamanian K.G. 2000. *Asparagus*. *In*: Wu Z.Y. and Raven P.H. (eds.), Flora of China **24**: 208–215. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Chinese Pharmacopoeia Commission 2020. Pharmacopoeia of the People's Republic of China 2020th edition. China Medical Science Press, Beijing.

Fukuda T., Ashizawa H., Suzuki R., Ochiai T., Nakamura T., Kanno A., Kameya T. and Yokoyama J. 2005. Molecular phylogeny of the genus *Asparagus* (*Asparagaceae*) inferred from plastid *petB* intron and *petD–rpoA* intergenic spacer sequences. Pl. Spec. Biol. **20**: 121–132.

Fukuda T., Song I.J., Ito T., Hayakawa H., Minamiya Y., Kanno A., Nakayama H. and Yokoyama J. 2011. Nucleotide sequence variations in a medicinal relative of *Asparagus*, *Asparagus cochinchinensis* (Lour.) Merrill (*Asparagaceae*). Amer. J. Pl. Sci. **2**: 765–767.

Hao J.D., Mei Q.X. and Yang H. (eds.) 2011. [Auxiliary explanation of Pharmacopoeia of the People's Republic of China 2010th edition, Part 1 (Chinese Materia Medica and Prepared Slices of Chinesrude Drugs)]. China Press of Traditional Chinese Medicine Co., Ltd., Beijing (in Chinese). 郝近大，梅全喜，杨华（編）2011. 中华人民共和国药典补助说明 2010 年版 一部（药材及饮片）. 中国中医药出版社，北京.

Huang L.Q., Jin Y., Yuan Y. and Peng H.S. (eds.) 2017. [The essence of the "gold" picture book of authentic crude drugs]. Shanghai Scientific & Technical Publishers, Shanghai (in Chinese). 黄璐琦，金艳，袁媛，彭华胜（編）2017. 道地药材 " 黄金 " 图谱精粹. 上海科学技术出版社，上海.

Kumar S., Stecher G., Li M., Knyaz C. and Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol.

35: 1547–1549.
Lee S.R., Park H.S., Kim B.Y., Lee J.H., Fan Q., Gaskin J.F. and Kim Y.D. 2019. An unexpected genetic diversity pattern and a complex demographic history of a rare medicinal herb, Chinese asparagus (*Asparagus cochinchinensis*) in Korea. Sci. Rep. **9**: 9757.
Lu S.D. 2009. [Asparagi radix continues to sell well and prices rise]. Modern Chin. Med. **11**(8): 44 (in Chinese). 陆善旦 2009. 天冬持续走俏价扬. 中国现代中药. **11**(8): 44.
Lu X.Y., Chen A.M., Liu D., Zhan R.Y., Tang H., Chen Y.C. and Yang X.D. 2019. Research Status and Prospects on Cultivation and Processing Technique of *Asparagus cochinchinensis* in Neijiang Area. Modern Agric. Sci. Technol. **2019**(14): 77–79 (in Chinese).
Ministry of Health Labour and Welfare of Japan 2021. The Japanese Pharmacopoeia Eighteenth Edition. Ministry of Health Labour and Welfare of Japan, Tokyo.
Mizukami H., Okabe Y., Kohda H. and Hiraoka N. 2000. Identification of the crude drug *Atractylodes* rhizome (Byaku-jutsu) and *Atractylodes lancea* rhizome (So-jutsu) using chloroplast *trnK* sequence as a molecular marker. Biol. Pharm. Bull. **23**: 589–594.
Namba T. 1993. The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese medicines) with Color Pictures, New Completely Revised Edition, vol. **1**. Hoikusha Publishing Co., Ltd., Tokyo (in Japanese).
Ou L.J., Zhang R.W., Tan Z.W., Zhao T.T. and She C.W. 2011. Nuclear DNA ITS sequence analysis of *Asparagus cochinchinensis* from different geographical regions in China. Chin. Tradit. Herbal Drugs **42**(7): 1402–1406 (in Chinese).
Shiba M., Kondo K., Miki E., Yamaji H., Morota T., Terabayashi S., Takeda S., Sasaki H., Miyamoto K. and Aburada M. 2006. Identification of medicinal *Atractylodes* based on ITS sequences of nrDNA. Biol. Pharm. Bull. **29**: 315–320.
Su B., Gao Z.Y., Que L., Gao Y.Z., Zhang X.L., Zhao R., Yang J. and Shi J.H. 2013. [Yunnan Famous and Special Medicinal Materials Planting Technology Series–Asparagi radix Tiandong]. Yunnan Science and Technology Press Co., Ltd., Kunming (in Chinese). 苏豹，高宗亚，却翎，高玉竹，张学莉，赵仁，杨静，施建辉 . 2013. 云南名特药材种植技术丛书 - 天冬. 云南科技出版社出版，昆明.
Taberlet P., Gielly L., Pautou G. and Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. **17**: 1105–1109.
Varshney R., Graner A. and Sorrells M. 2005. Genetic microsatellite markers in plants: features and applications. Trends Biotechnol. **23**: 48–55.
Vieira M., Santini L., Diniz A. and Munhoz C. 2016. Microsatellite markers: what they mean and why they are so helpful. Genet. Mol. Biol. **39**: 312–328.
White T.J., Bruns T., Lee S. and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis M.A., Gelfand D.H., Sninsky J.J. and White T.J. (eds.), PCR Protocols, A Guide to Methods and Applications. Pp. 315–322. Academic Press, San Diego.
Xiao P.G. 2002. Tiandong *In*: Xiao P.G., Li D.P. and Yang S.L. (eds.), Modern Chinese Materia Medica, vol. **1**. Chemical Industry Press, Beijing (in Chinese).
Xie Y.Y. 2019. [Michi Tianmendong] *Asparagus meioclados* H. Lévl. *In*: Huang L.Q. and Ma X.J. (eds.), Endemic Species of Chinese Medicinal Plants. Pp. 278–279. People's Medical Publishing House, Beijing (in Chinese). 谢月英 2019. 密齿天门冬. 黄璐琦，马小军（主编）中国药用植物特有种. Pp. 278–279. 人民卫生出版社，北京.
Yamaji H., Kondo K., Miki E., Iketani H., Yamaguchi M. and Takeda O. 2009. Discrimination of Xingren from seeds of *Prunus* st. *Armeniaca* species (*Rosaceae*) by partial *rpl16* intron sequences of cpDNA, and the botanical origin of Xingrens in markets in Japan. J. Jap. Bot. **84**: 85–91.
Zhang T.Y. and Qin S.Y. 1992a. [Historical study of Asparagi radix on the Materia Medica]. Zhong yao cai **1992**(12): 43–44 (in Chinese). 张天友，秦松云 1992a 天门冬的本草考证. 中药材 **1992**(12): 43–44.
Zhang T.Y. and Qin S.Y. 1992b. Study on the Botanical Resources of Asparaguses in Sichuan Province. Resource Development and Protection **1992**(4): 268–269 (in Chinese).
Zhu S., Fushimi H., Cai S. and Komatsu K. 2003. Phylogenetic relationship in the genus *Panax*: inferred from chloroplast *trnK* gene and nuclear 18S rRNA gene sequences. Planta Med. **69**: 647–653.

Appendix 1

Plant samples of *Asparagus* species and outgroups used for molecular phylogenetic analyses. Species name, collection area or source, voucher specimen, and DDBJ/GenBank accession numbers (in parentheses) are provided. Species are alphabetically ordered, and species with swollen tuberous root are underlined. Newly generated sequences of the ITS regions and *trnL-trnF* intergenic spacer are indicated by asterisk (*) and plus (+), respectively. The other accessions are those of the ITS regions.

Asparagus aethiopicus L., unknown, CU/BSU/PSS02 (KJ868763). ***A. asparagoides*** (L.) Druce, unknown (KJ868770). ***A. cochinchinensis*** (Lour.) Merr., CHINA, Hubei, Enshi-zhou, Lichuan-xian, wild, THS95139 (LC766390*, LC766391*); ibid., THS95140 (LC766392*, LC766393*); ibid., THS95141 (LC766394*, LC766395*); ibid., THS104656 (LC766396*, LC766397*); CHINA, Hubei, Enshi-zhou, Xuan'en-xian, wild, THS104653 (LC766398*, LC766399*); ibid., THS95142 (LC766408*,

LC766409*); ibid., THS95143 (LC766410*, LC766411*); ibid., THS95144 (LC766412*, LC766413*); ibid., THS95145 (LC766414*, LC766415*); CHINA, Hubei, Enshi-zhou, Xianfeng-xian, wild, THS95135 (LC766400*, LC766401*); ibid., THS95136 (LC766402*, LC766403*); ibid., THS95137 (LC766404*, LC766405*); ibid., THS95138 (LC766406*, LC766407*); CHINA, Hubei, Jingmen-shi, Jingshan-shi, wild, THS95150 (LC766416*, LC766417*); ibid., THS95151 (LC766418*, LC766419*); CHINA, Guangdong, Chaozhou-shi, Chao'an-xian, wild, THS104657 (LC766420*, LC766421*, LC766422*); CHINA, Guangdong, Shenzhen-shi, Longgang-qu, wild, THS104652 (LC766423*, LC766424*); CHINA, Guangxi, Yulin-shi, Fumian-qu, cultivated, THS98357 (LC766425*, LC766426*); ibid., THS104654 (LC766427*, LC766428*); ibid., THS104655 (LC766429*, LC766430*); CHINA, Chongqing, Nanchuan-qu, cultivated, THS80092 (LC766431*, LC766432*); CHINA, Sichuan, Guangyuan-shi, Xhaohua-qu, wild, THS104658 (LC766433*, LC766434*); CHINA, Sichuan, Mian'yang-shi, Pingwu-xian, wild, THS95133 (LC766435*, LC766436*); ibid., THS95134 (LC766437*, LC766438*); CHINA, Sichuan, Neijiang-shi, Dongxing-qu, cultivated, THS106516 (LC766439*, LC766440*); ibid., THS81132 (LC766441*, LC766442*); ibid., THS81133 (LC766443*, LC766444*); CHINA, Sichuan, Yibin-shi, Pingshan-xian, wild, THS95130 (LC766445*, LC766446*); ibid., THS95131 (LC766447*, LC766448*); CHINA, Guizhou, Guiyang-shi, Xiuwen-xian, wild, THS95119 (LC766449*, LC766450*); ibid., THS95120 (LC766451*, LC766452*); ibid., THS95121 (LC766453*, LC766454*); ibid., THS95122 (LC766455*, LC766456*); ibid., THS95123 (LC766457*, LC766458*); CHINA, Guizhou, Zunyi-shi, Huichuan-qu, wild, THS95126 (LC766459*, LC766460*); ibid., THS95127 (LC766461*, LC766462*); CHINA, Guizhou, Zunyi-shi, Renhuai-shi, wild, THS95128 (LC766463*, LC766464*); ibid., THS95129 (LC766465*, LC766466*); JAPAN, Okinawa, Miyakojima-shi, maintained in Tsumura Botanical Garden, THS80091 (LC766467*, LC766468*); maintained in Tsukuba Division, Research Center for Medicinal Plant Resources, National Institutes of Biomedical Innovation, Health and Nutrition, THS80093 (LC766469*, LC766470*); unknown, PS0057MT01 (FJ980278); unknown, ChoSH-001 (JN171595). ***A. densiflorus*** (Kunth) Jessop, maintained in Guangzhou University of Chinese Medicine, THS101518 (LC766580*); ibid., THS101519 (LC766581*, LC766582*). ***A. falcatus*** L., unknown (MH048066). ***A. filicinus*** Buch.-Ham. ex D.Don, CHINA, Yunnan, Lijiang-shi, Ninglang-xian, wild, THS104661 (OR852571, LC766576*, LC766577*); CHINA, Yunnan, Zhaotong-shi, Yiliang-xian, wild, THS82448 (LC766578*). ***A. gonoclados*** Baker, unknown (MH048067). ***A. lycopodineus*** (Baker) F.T.Wang & Tang, CHINA, Sichuan, Emeishan-shi, wild, THS104702 (LC766520*, LC766521*); ibid., THS104703 (LC766522*, LC766523*); maintained in Kunming Botanical Garden, THS88427 (LC766524*, LC766525*); ibid., THS88428 (LC766526*, LC766527*); unknown (GQ166855). ***A. meioclados*** H.Lév., CHINA, Yunnan, Baoshan-shi, Tengchong-xian, wild, THS81033 (LC766544*); CHINA, Yunnan, Lijiang-shi, Ninglang-xian, wild, THS104662 (LC766548*, LC766549*); ibid., THS104663 (LC766550*, LC766551*); ibid., THS104664 (LC766552*, LC766553*); CHINA, Yunnan, Wenshan-zhou, Wenshan-shi, wild, THS82434 (LC766554*, LC766555*); CHINA, Yunnan, Yuxi-shi, Xinping-xian, wild, THS104698 (LC766556*, LC766557*); ibid., THS104699 (LC766558*, LC766559*); CHINA, Yunnan, Yuxi-shi, Yuanjiang-xian, wild, THS104696 (LC766560*, LC766561*); ibid., THS104697 (LC766562*, LC766563*); ***A. myriacanthus*** F.T.Wang & S.C.Chen, CHINA, Yunnan, Diqing-zhou, Deqin-xian, KUN 0179436 (LC766579*). ***A. munitus*** F.T.Wang & S.C.Chen, CHINA, Sichuan, Liangshan-zhou, Mulixian, wild, THS104665 (LC766511*, LC766512*); ibid., THS104666 (LC766513*, LC766514*); ibid., THS104667 (LC766515*, LC766516*); ibid., THS104668 (LC766517*, LC767102+); ibid., THS104669 (LC766518*, LC767103+); ibid., THS104670 (LC766519*, LC767104+). ***A. officinalis*** L., JAPAN, Yamanashi, Hokuto-shi, cultivated, THS78658 (LC766585*, LC766586*). ***A. racemosus*** Willd., NEPAL, Daman Makwanpur, THS101830 (LC766587*, LC766588*); JAPAN, Okinawa, Ishigaki-shi, THS102300 (LC766589*, LC766590*); unknown (KR215620); India, unknown (MK513793). ***A. retrofractus*** L., unknown (KJ868766). ***A. schoberioides*** Kunth, unknown, LX4 (KX421721). ***A. setaceus*** (Kunth) Jessop, maintained in Kunming Botanical Garden, THS104519 (LC766583*, LC766584*). ***A. subscandens*** F.T.Wang & S.C.Chen, CHINA, Yunnan, Puer-shi, Simao-qu, wild, THS104685 (LC766528*, LC766529*); ibid., THS104686 (LC766530*, LC766531*); ibid., THS104687 (LC766532*, LC766533*); ibid., THS104688 (LC766534*, LC766535*); CHINA, Yunnan, Xishuangbanna-zhou, Jinghong-shi, wild, THS104671 (LC766536*, LC766537*); CHINA, Yunnan, Xishuangbanna-zhou, Menghai-xian, wild, THS104672 (LC766538*, LC766539*), MYANMAR, Kayah State, Loikaw District, wild, TNS1317313 (LC766540*, LC766541*), MYANMAR, Shan State, Taunggyi District, wild, TNS1329184 (LC766542*, LC766543*). ***A. taliensis*** F.T.Wang & Tang ex S.C.Chen, CHINA, Guizhou, Zunyi-shi, Wuchuan-xian, cultivated, THS106513 (LC766471*, LC766472*); CHINA, Yunnan, Dali-zhou, Binchuan-xian, cultivated, THS104675 (LC766473*, LC766474*); CHINA, Yunnan, Dali-zhou, Xiangyun-xian, wild, THS106514 (LC766475*, LC767087+); CHINA, Yunnan, Kunming-shi, Panlong-qu, cultivated,

THS88429 (LC766476*, LC766477*, LC767088+), cultivated, THS88430 (LC766478*, LC766479*, LC767089+); CHINA, Yunnan, Lijiang-shi, Ninglang-xian, cultivated, THS104677 (LC766480*, LC766481*); CHINA, Yunnan, Lijiang-shi, Yongsheng-xian, wild, THS91111 (LC766493*, LC766494*, LC767095+); ibid., THS91112 (LC766495*, LC766496*, LC767096+); ibid., THS91115 (LC766501*, LC766502*, LC767099+); ibid., THS91116 (LC766503*, LC766504*, LC767100+), cultivated, THS106515 (LC766482*, LC767090+); ibid., THS104676 (LC766483*, LC766484*); ibid., THS86972 (LC766485*, LC766486*, LC767091+); ibid., THS91108 (LC766487*, LC766488*, LC767092+); ibid., THS91109 (LC766489*, LC766490*, LC767093+); ibid., THS91110 (LC766491*, LC766492*, LC767094+); ibid., THS91113 (LC766497*, LC766498*, LC767097+); ibid., THS91114 (LC766499*, LC766500*, LC767098+); ibid., THS92022 (LC766505*, LC766506*, LC767101+); ibid., THS92023 (LC766507*, LC766508*); CHINA, Yunnan, Wenshan-zhou, Wenshan-shi, wild, THS104674 (LC766509*, LC766510*). ***A. trichoclados*** (F.T.Wang & Tang) F.T.Wang & S.C.Chen, CHINA, Yunnan, Lincang-shi, Linxiang-qu, wild, THS104705 (LC766564*, LC766565*); ibid., THS104706 (LC766566*, LC766567*); ibid., THS104707 (LC766568*, LC766569*); ibid., THS104708 (LC766570*, LC766571*); CHINA, Yunnan, Puer-shi, Zhenyuan-xian, THS104709 (LC766572*, LC766573*). ***A. trichophyllus*** Bunge, CHINA, Hebei, Ge131180 (MH712667). ***A. tibeticus*** F.T.Wang & S.C.Chen, CHINA, Xizang, Lasa-shi, wild, THS104681 (LC766591*, LC766592*); ibid., THS104682 (LC766593*, LC766594*); ibid., THS104683 (LC766595*, LC766596*). ***Chlorogalum parviflorum*** S.Watson, unknown, Sanders 30293 [UCR 154983] (KP008327). ***Hesperaloe campanulata*** G.D.Starr, unknown, Starr 93-001 [ARIZ 319675] (KP008345). ***Hesperoyucca whipplei*** (Torr.) Baker ex Trel., unknown, Vanderplank and Arauz 1303191 [SD] (KP008362). ***Schoenolirion albiflorum*** R.R.Gates, unknown, Scanlon 405 [FLAS 208612] (KP008364).

Appendix 2

Crude drug samples of Asparagi radix in medicinal markets in China and Japan. Species name identified in this study (bold, italic, underlined), market area (bold), collection year (bold), source locality, wild/cultivated/unknown, voucher specimen, and number of individuals obtained with DDBJ accession numbers (in parentheses) are provided. Species are alphabetically ordered. Sequences of *trnL-trnF* intergenic spacer are indicated by plus (+).

Asparagus cochinchinensis, **Anhui**, **Bozhou**, **2020**: China, Guangxi, Yulin-shi, cultivated, THS103321 (n=3, 1: LC766630, LC766631, 2: LC766632, OR852583, LC766633, 3: LC766634, LC766635); China, Guizhou, wild, THS103314 (n = 3, 1: LC766687, LC766688, 2: LC766689, LC766690, LC766691, 3: LC766696, LC766697, LC766698); China, Guizhou, Qiannan-zhou, wild, THS103317 (n = 3, 1: LC766787, LC766788, 2: LC766789, LC766790, LC766791, 3: LC766792, LC766793); **Guangxi**, **Yulin**, **2019**: China, Guangxi, cultivated, THS102034 (n = 3, 1: LC766599, LC766600, LC766601, 2: LC766597, LC766598, 3: LC766602, LC766603); China, Guangxi, wild, THS102033 (n = 2, 1: LC766616, LC766617, 2: LC766618, LC766619); ibid., THS102039 (n = 3, 1: LC766620, LC766621, 2: LC766622, LC766623, 3: LC766624, LC766625); China, Guangxi, Yulin-shi, cultivated, THS102027 (n = 3, 1: LC766636, LC766637, 2: LC766638, LC766639, 3: LC766640, LC766641); ibid., THS102028 (n = 3, 1: LC766642, LC766643, 2: LC766644, LC766645, 3: LC766646, LC766647); ibid., THS102029 (n = 3, 1: LC766648, LC766649, 2: LC766650, LC766651, 3: LC766652, LC766653); ibid., THS102036 (n = 3, 1: LC766654, LC766655, 2: LC766656, LC766657, 3: LC766658, LC766659); ibid., THS102038 (n = 3, 1: LC766660, LC766661, 2: LC766662, LC766663, 3: LC766664, LC766665); China, Guizhou, wild, THS102031 (n = 3, 1: LC766692, LC766693, 2: LC766694, LC766695, 3: LC766699, LC766700); ibid., THS102037 (n = 1, LC766701, LC766702); ibid., THS102041 (n = 2, 1: LC766703, LC766704, 2: LC766709, OR852589); **Hebei**, **Anguo**, **2020**: China, Chongqing, wild, THS103642 (n = 3, 1: LC766666, LC766667, 2: LC766668, LC766669, 3: LC766670, LC766671, OR852587); China, Guizhou, wild, THS103633 (n = 3, 1: LC766707, LC766708, OR852588, 2: LC766705, LC766706, 3: LC766710, LC766711); ibid., THS103636 (n = 5, 1: LC766714, LC766715, OR852590, 2: LC766716, LC766717, OR852591, 3: LC766718, LC766719, LC766720, 4: LC766712, LC766713, 5: LC766729, LC766730, OR852592); ibid., THS103639 (n = 3, 1: LC766721, LC766722, 3: LC766725, LC766726, 4: LC766727, LC766728); ibid., THS103641 (n = 3, 1: LC766739, LC766740, OR852593, 2: LC766736, LC766737, LC766738, 3: LC766741, LC766742); China, Guizhou, Zunyi-shi, Renhuai-shi , wild, THS103634 (n = 5, 1: LC766794, LC766795, 2: LC766796, LC766797, 3: LC766798, LC766799, 4: LC766800, LC766801, 5: OR852579, LC766806, OR852596); China, Yunnan, cultivated, THS103638 (n = 3, 1: LC766823, LC766824, OR852597, 2: LC766821, LC766822, 3: LC766825, LC766826); China, Yunnan, wild, THS103645 (n = 4, 2: LC766906, LC766907, 3: LC766908, LC766909, OR852607, 4: LC766910, LC766911, OR852608, 5: OR852580, LC766922, OR852609); **Sichuan**, **Hehuachi**, **2014**: China, Sichuan, unknown, THS92093 (n = 1, LC766672); ibid., THS92094 (n = 2, 1: LC766673, 2: OR852584, LC766674); China, Sichuan, Aba-zhou,

unknown, THS92092 (n = 1, LC766675); China, Sichuan, Wanyuan-shi, unknown, THS92091 (n = 2, 1: LC766677, 2: LC766680); 2019: China, Guangxi, cultivated, THS102015 (n = 3, 1: LC766604, LC766605, 2: LC766606, LC766607, 3: LC766608, LC766609); ibid., THS102016 (n = 3, 1: LC766610, LC766611, 2: LC766612, LC766613, 3: LC766614, LC766615); China, Guangxi, Guigang, cultivated, THS102022 (n = 3, 1: LC766626, LC766627, 2: OR852572, LC766628, 3: OR852573, LC766629); China, Guizhou, wild, THS102017 (n = 3, 1: LC766743, LC766744, 2: OR852575, LC766745, 3: LC766746, LC766747); ibid., THS102018 (n = 1, 1: LC766748, LC766749); ibid., THS102019 (n = 3, 1: LC766754, LC766755, 2: LC766756, LC766757, 3: LC766764, LC766765, OR852594); ibid., THS102020 (n = 3, 1: LC766758, LC766759, LC766760, 2: LC766761, LC766762, LC766763, 3: LC766766, LC766767); ibid., THS102021 (n = 3, 1: OR852576, LC766768, LC766769, 2: OR852577, LC766770, LC766771, 3: OR852578, LC766776, LC766777); ibid., THS102026 (n = 3, 1: LC766772, LC766773, 2: LC766774, LC766775, 3: LC766778, LC766779); China, Guizhou, Zunyi-shi, Xishui-xian, wild, THS102023 (n = 3, 1: LC766780, LC766781, LC766782, 2: LC766783, LC766784, OR852595, 3: LC766785, LC766786); **Japan**, **2010**: China, Guizhou, unknown, THS100697 (n = 1, LC767003, LC767004) ; ibid., THS100698 (n = 1, LC767005, LC767006); ibid., THS100699 (n = 1, LC767007); 2011: China, Guizhou, unknown, THS100694 (n = 1, LC767008, LC767009); ibid., THS100695 (n = 1, LC767010, LC767011); ibid., THS100696 (n = 1, LC767013, LC767014, OR852612); 2013: China, Shaanxi, unknown, THS100691 (n = 1, LC767075, LC767076); ibid., THS100692 (n = 1, LC767077, LC767078); ibid., THS100693 (n = 1, LC767079, LC767080); 2014: China, Shaanxi, unknown, THS100688 (n = 1, LC767081, LC767082); ibid., THS100689 (n = 1, LC767083, LC767084); ibid., THS100690 (n = 1, LC767085, LC767086); 2016: China, Yunnan, unknown, THS100682 (n = 1, LC767024, LC767025); ibid., THS100683 (n = 1, LC767026); ibid., THS100684 (n = 1, LC767027, LC767028); 2017: China, Hubei, unknown, THS100752 (n = 2, 1: LC766984, 3: LC766988, LC766989); ibid., THS100753 (n = 3, 1: LC766990, LC766991, 2: LC766992, LC766993, 3: LC766994, LC766995); ibid., THS100754 (n = 3, 1: LC766996, LC766997, 2: LC766998, LC766999, 3: LC767000, LC767001, LC767002); China, Yunnan, unknown, THS100679 (n = 1, LC767029); ibid., THS100680 (n = 1, LC767030, LC767031); ibid., THS100681 (n = 1, LC767032, LC767033); 2018: China, Guizhou, unknown, THS100748 (n = 3, 1: LC767012, 2: LC767015, LC767016, LC767017, 4: LC767018, LC767019); China, Yunnan, unknown, THS100676 (n = 1, LC767034, LC767035); ibid., THS100677 (n = 1, LC767036, LC767037); ibid., THS100678 (n = 1, LC767038, LC767039). ***A. lycopodineus***, **Sichuan**, **Hehuachi**, **2019**: China, Sichuan, Leshan-shi or Yibin-shi, wild, THS102025 (n = 2, 2: OR852574, LC766676, 3: LC766678, LC766679); China, Sichuan, Yibin-shi, wild, THS102032 (n = 3, 1: LC766681, LC766682, 2: LC766683, LC766684, 3: LC766685, LC766686). ***A. meioclados*** or ***A. trichoclados***, **Yunnan**, **Xinluosi**, 2014: China, Yunnan, Dali-zhou, unknown, THS92088 (n = 1, LC766931, LC766932). ***A. subscandens***, **Anhui**, **Bozhou**, **2020**: China, Yunnan, unknown, THS103325 (n = 3, 1: LC766827, LC766828, 2: LC766829, LC766830, 3: LC766831, LC766832); China, Yunnan, wild, THS103312 (n = 2, 1: LC766833, LC766834, 3: LC766835, LC766836, LC766837); ibid., THS103313 (n = 3, 1: LC766838, LC766839, LC766840, 2: LC766841, LC766842, LC766843, 3: LC766844, LC766845); ibid., THS103319 (n = 3, 1: LC766846, LC766847, 2: LC766848, LC766849, LC766850, 3: LC766851, LC766852, LC766853); China, Yunnan, Lincang-shi, unknown, THS103316 (n = 2, 2: OR852581, LC766963, LC766964, 3: LC766967, LC766968, LC766969, LC766970); China, Yunnan, Lincang-shi, wild, THS103318 (n = 3, 1: LC766949, LC766950, LC766951, 2: LC766952, LC766953, LC766954, 3: LC766957, LC766958, LC766959); ibid., THS103324 (n = 2, 1: LC766955, LC766956, 2: LC766960, LC766961, LC766962); **Guangxi**, **Yulin**, **2019**: China, Yunnan, wild, THS102035 (n = 3, 1: LC766854, LC766855, 2: LC766856, LC766857, 3: LC766858, LC766859); Myanmar, wild, THS102030 (n = 2, 1: LC766976, LC766977, 3: LC766982, LC766983, OR852611, OR852586); ibid., THS102040 (n = 3, 1: LC766978, LC766979, 2: LC766980, LC766981, 3: LC766985, LC766986, LC766987); **Hebei**, **Anguo**, **2020**: China, Guizhou, wild, THS103639 (n = 2, 2: LC766723, LC766724, 5: LC766731, LC766732); ibid., THS103640 (n = 1, LC766733, LC766734, LC766735); China, Yunnan, wild, THS103632 (n = 5, 1: LC766864, LC766865, OR852598, 2: LC766866, LC766867, OR852599, 3: LC766860, LC766861, 4: LC766862, LC766863, 5: LC766874, LC766875, OR852600); ibid., THS103635 (n = 7, 1: LC766868, LC766869, 2: LC766876, LC766877, OR852601, 3: LC766870, LC766871, 4: LC766872, LC766873, 5: LC766878, LC766879, OR852602, 6: LC766880, LC766881, OR852603, 7: LC766886, LC766887, OR852604); ibid., THS103637 (n = 5, 1: LC766882, LC766883, 2: LC766884, LC766885, 3: LC766888, LC766889, OR852605, 4: LC766890, LC766891, OR852606, 5: LC766892, LC766893); ibid., THS103643 (n = 3, 1: LC766894, LC766895, 2: LC766896, LC766897, 3: LC766898, LC766899); ibid., THS103646 (n = 6, 1: LC766912, LC766913, 2: LC766920, LC766921,

OR852610, 3: LC766914, LC766915, 5: LC766916, LC766917, 6: LC766918, LC766919, 7: LC766923, LC766924); **Sichuan**, **Hehuachi**, **2019**: China, Yunnan, Dali-zhou, wild, THS102024 (n = 3, 1: LC766935, LC766936, LC766937, 2: LC766933, LC766934, 3: LC766938, LC766939); **Japan**, **2015**: China, Yunnan, unknown, THS100685 (n = 1, LC767020, LC767021); ibid., THS100686 (n = 1, LC767022, LC767023). ***A. taliensis***, **Anhui**, **Bozhou**, 2020: China, Yunnan, cultivated, THS103315 (n = 3, 1: LC766802, LC766803, 2: LC766804, LC766805, 3: LC766807, LC766808, 1: LC767106^{+}, 2: LC767107^{+}, 3: LC767108^{+}); ibid., THS103320 (n = 3, 1: LC766809, LC766810, 2: LC766811, LC766812, 3: LC766813, LC766814, 1: LC767109^{+}, 2: LC767110^{+}, 3: LC767111^{+}); ibid., THS103326 (n = 3, 1: LC766815, LC766816, 2: LC766817, LC766818, 3: LC766819, LC766820, 1: LC767112^{+}, 2: LC767113^{+}, 3: LC767114^{+}); China, Yunnan, Lijiang-shi, cultivated, THS103323 (n = 3, 1: LC766942, LC766943, OR852585, 2: LC766940, LC766941, 3: LC766945, LC766946, 1: LC767121^{+}, 2: LC767122^{+}, 3: LC767123^{+}); China, Yunnan, Puer-shi, cultivated, THS103322 (n = 3, 1: LC766971, LC766972, LC766973, 2: LC766965, LC766966, 3: LC766974, LC766975, 1: LC767124^{+}, 2: LC767125^{+}, 3: LC767126^{+}); **Hebei**, **Anguo**, **2020**: China, Yunnan, wild, THS103644 (n = 3, 1: LC766900, LC766901, 2: LC766902, LC766903, 3: LC766904, LC766905, 1: LC767115^{+}, 2: LC767116^{+}, 3: LC767117^{+}); **Sichuan**, **Hehuachi**, **2019**: China, Guizhou, wild, THS102018 (n = 2, 2: LC766750, LC766751, 3: LC766752, LC766753, 2: LC767105^{+}); China, Yunnan, Dali-zhou, cultivated, THS102014 (n = 3, 1: LC766925, LC766926, 2: LC766927, LC766928, 3: LC766929, LC766930, 1: LC767118^{+}, 2: LC767119^{+}, 3: LC767120^{+}); **Yunnan**, **Xinluosi**, **2014**: China, Yunnan, Lijiang-shi, unknown, THS92089 (n = 1, 1: LC766947, LC766948); **Japan**, **2018**: China, Yunnan, unknown, THS100755 (n = 3, 1: LC767040, LC767041, 2: LC767042, LC767043, 3: LC767044, LC767045, 1: LC767127^{+}, 2: LC767128^{+}, 3: LC767129^{+}); ibid., THS100756 (n = 3, 1: LC767046, LC767047, 2: LC767048, LC767049, 3: LC767054, OR852582, LC767055, 1: LC767130^{+}, 2: LC767131^{+}, 3: LC767132^{+}); ibid., THS100757 (n = 2, 1: LC767050, LC767051, 2: LC767056, LC767057, 1: LC767133^{+}, 2: LC767134^{+}); ibid., THS100758 (n = 3, 1: LC767058, LC767059, 2: LC767060, LC767061, 3: LC767062, LC767063, 1: LC767135^{+}, LC767136^{+}, LC767137^{+}); ibid., THS100759 (n = 2, 1: LC767064, LC767065, 2: LC767066, LC767067, 1: LC767138^{+}, 2: LC767139^{+}); ibid., THS100760 (n = 2, 1: LC767070, LC767071, LC767072, 3: LC767073, LC767074, 1: LC767140^{+}, 3: LC767141^{+}).

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日本および中国の市場に流通する生薬材テンモンドウ（天門冬）の基原植物の種レベルの多様性を明らかにするために，クサスギカズラ *Asparagus cochinchinensis* とその近縁種の種鑑別用 DNA マーカーを開発し，市場調査を行った．開発のために *Asparagus* 属 21 種，107 サンプルの ITS 領域の塩基配列を決定し，一部の種については葉緑体 DNA の *trnL-trnF* 遺伝子間領域も調査した．その結果，*A. meioclados* と *A. trichoclados* を除いて，調査した種は互いに区別することができた．テンモンドウの日中市場流通品 238 サンプルについて DNA 鑑定を行った結果，最も多い基原種は *A. cochinchinensis* (56%), 続いて *A. subscandens* (25%), *A. taliensis* (16%), *A. lycopodineus* (2%), および *A. meioclados* または *A. trichoclados* (0.4%) であった．中国市場からはこれら全種が見い出され，日本市場からは *A. cochinchinensis*, *A. taliensis*, *A. subscandens* の 3 種が認められた．栽培品として販売される市場品からは，*A. cochinchinensis*, *A. taliensis* のみが見い出された．*A. subscandens* と判定された生薬のうち多くは中国産と表記されていたが，ミャンマー産 *A. subscandens* と DNA 型が類似していたことから，それらがミャンマーから輸入されていると推定された．

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