ISSN : 1225-9918
Journal of Life Science 2012 Vol. 22. No. 3. 285~292 DOI : http://dx.doi.org/10.5352/JLS.2012.22.3.285

Analysis of the Phylogenetic Relationships in the Genus *Spiraea* Based on the Nuclear Ribosomal DNA ITS Region

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Received November 19, 2011 / Revised February 23, 2012 / Accepted February 27, 2012

Genus *Spiraea* is composed of many long-lived woody species that are primarily distributed throughout Asia and Europe. In this study, we evaluated a representative sample of the 38 taxa in the world, including 14 in Korea, with nuclear ribosomal DNA internal transcribed spacer sequences (ITS) to estimate genetic relationships within the genus. The molecular data allowed us to resolve well-supported clades in the taxa. In 47 world accessions (38 taxa: 14 Korean taxa, 33 world taxa, and 9 overlapping taxa), total alignment length was 689 positions, of which 452 were parsimony informative, 527 variable, 75 singleton, and 159 constant characters. Although the phylogenic tree showed that many taxa of genus *Spiraea* were well separated from each other, many branches were not congruent with the morphological characteristics and geographical distributions of the genus. There were 430 segregating sites and the nucleotide diversity (π) value was 0.281. Under the neutral mutation hypothesis, the probability that the Tajima test statistic (D) is positive (2.325) is more than 0.5. Therefore, there may be a site at which natural selection, which increases genetic variation, is operating.

Key words : Genus *Spiraea*, nuclear ribosomal DNA internal transcribed spacer sequences (ITS), phylogenic tree

Introduction

The genus *Spiraea* belongs to one of the family *Rosaceae*. These are deciduous shrubs 0.2 to 2.5 m in height. The genus includes about 90 species, which grow mostly in forest-steppe, steppe, semi-dessert zones, and in subalpine belt of the mountains of the North Hemisphere [12]. In Asia, the southern border runs through the eastern and northern Himalayas, and in America, through the central part of Mexico [19].

Spiraea has been variously divided by different authors into subgenera, sections, series, and cycles [12]. Inflorescence morphology has been emphasized in most of these groupings, as reflected in the widely accepted classification followed by Rehder [13]: *Spiraria* Ser. (=*Spirace*) with panicles, *Calospira* with compound corymbs, and *Chamaedryon* with simple corymbiform or umbellate inflorescences.

Traditionally, many taxa have been described within the complex on the basis of difference in leaf size and shape, and other morphological traits [3]. Morphologically, the complex is very diverse with diagnostic characters separating varieties combined in different ways within it, resulting in taxonomic confusion and difficulty in determining boundaries of varieties [8,19]. Taxonomic problems are further compounded because some varieties, especially taxa in Korea, are geographically sympatric and appear to hybridize readily [9].

Determination of DNA sequence heterogeneity can provide useful taxonomic information on intra- and inter-specific genetic variation in plants. Nuclear ribosomal DNA internal transcribed spacer sequences (ITS) is eukaryotic ribosomal RNA genes (known as ribosomal DNA or rDNA) are found as parts of repeat units that are arranged in tandem arrays, located at the chromosomal sites known as nuclear organizing regions (NORs). Each repeat unit consists of a transcribed region (having genes for 18S, 5.8S and 26S rRNAs and the external transcribed spacers i.e. ETS1 and ETS2) and a non-transcribed spacer (NTS) region. In the transcribed region, internal transcribed spacers (ITS) are found on either side of 5.8S rRNA gene and are described as ITS1 and ITS2. The two internal transcribed spacers (ITS1 and ITS2) have been shown to be relatively valuable targets for defining markers in systematic studies [18]. In fact, ITS1 and ITS2 have proven useful for resolving phylogenies of closely related taxa that have diverged relatively recently (<50 million years ago) and are excellent markers for species distinction, since they are relatively fast evolving sequences.

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Universal PCR primers designed from highly conserved regions flanking the ITS and its relatively small size (600-700 bp) enable easy amplification of ITS region due to high copy number (up to-30000 per cell) of rDNA repeats [3].

The purpose of this research is to do molecular data support the current classification of the species within the genus *Spiraea* in Korea. In addition, molecular evidence assumes an important role in phylogenetic reconstruction of species in this genus. I recommend the necessity of accuracy sequences of the genus *Spiraea* in GenBank according to species identification.

Materials and Methods

DNA extraction

Fourteen taxa of genus *Spiraea* were collected from large effective populations in Korea (Table 1) and identified according to Kim [4], Lee [7], and Kim and Sun [5]. Total genomic DNA was extracted from 0.5-1.0 g of fresh leaf material. A species of the closely related genus *Stephanandra* (*Stephanandra incise* (Thunb) Zabel) was included in the phylogenetic reconstruction as an outgroup.

DNA was extracted using the plant DNA Zol Kit (Life Technologies Inc., Grand Island, New York, USA) according to the manufacturer's protocol. Genomic DNA was stored at 4℃. DNA was checked for shearing and concentration by agarose electrophoresis and DyNA 200 fluorometer (Amersham Pharmacia Biotech, USA), respectively.

ITS analysis

Primer sets of about 20 bases in length (ITS1 and ITS2) [20] were used for PCR analysis (Table 2). These primers were based on well-characteristic DNA Sequences and were designed making use of conserved regions of the 18S and 28S rRNA genes to amplify the noncoding regions between the (ITS1 and ITS2) and 5.8S rRNA gene.

PCR amplifications were carried out in volumes of 25 ml using a PTC-100 DNA Engine Dyad Peltier thermal cycler (MJ Research, Watertown, MA, USA). Each reaction contained 50 ng of genomic DNA, 3 mm $MgCl_2$, 0.12 mm of each dNTP, 12.5 pmol of each primer, 0.25 units of BIOTAQ DNA polymerase (Bioline) and the associated NH_4 buffer at 13 concentrations. An initial denaturation step of 5 min at 94℃ was followed by 30 cycles of amplification (30 sec at 94℃, 30 sec at 42℃, 1 min at 72℃) and a final elongation step of 10 min at 72℃.

PCR products were separated on 1.5% agarose gels and purified using the QIAquick Gel Extraction Kit (QIAGEN). The amplified fragments were cloned into a bluescript vector (Agilent Technologies, USA) and sequenced using ABI Prism 377 Sequencer (Applied Biosystem, USA). At least ten individuals' clones of each taxon were analyzed.

Phylogenetic analysis

Disparity index was calculated a simple statistic to measure and test the homogeneity of substitution patterns between molecular sequences [6]. Estimates of evolutionary divergence between sequences were conducted using the maximum composite likelihood model [16].

Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information

Table 1. The collection sites and sections of genus *Spireae* in Korea

Scientific name	Localities	Section
S. blumei D. Don.	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do	*Chamaedryon* Ser.
S. cantoniensis D. Don.	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do	*Chamaedryon* Ser.
S. chamaedryfolia var. *ulmifolia* (Scop.) Maxim.	Sohol-eup, Pocheon-si, Gyeonggi-do	*Chamaedryon* Ser.
S. chartacea Nakai	Hongdo, Jindo-gun, Jeollanam-do	*Chamaedryon* Ser.
S. chinensis Maxim.	Dongdaemun-gu, Seoul	*Chamaedryon* Ser.
S. fritschiana var. *obtusifolia* Nakai	Sohol-eup, Pocheon-si, Gyeonggi-do	*Calospira* K. Koch.
S. fritschiana Schneid	Mt. Nayeon, Pohang-si, Gyeongsangbuk-do	*Calospira* K. Koch.
S. japonica L.	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do	*Chamaedryon* Ser.
S. miyabei Koidz.	Sucheon-dong, Osan-si, Gyeonggi-do	*Calospira* K. Koch.
S. prunifolia for. *simpliciflora* Nakai	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do	*Chamaedryon* Ser.
S. pubescens Turcz.	Mt. Gaya, Hapcheon-gun, Gyeongsangnam-do	*Chamaedryon* Ser.
S. salicifolia L.	Sohol-eup, Pocheon-si, Gyeonggi-do	Section *Spiraria*
S. thunbergii Sieb.	Sohol-eup, Pocheon-si, Gyeonggi-do	*Chamaedryon* Ser.
S. trichocarpa Nakai	Sucheon-dong, Osan-si, Gyeonggi-do	*Chamaedryon* Ser.

Criterion, corrected), Maximum Likelihood value (*lnL*), and the number of parameters (including branch lengths) are also presented. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+*G*) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+*I*). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well.

Codon-based tests of neutrality for analysis between ITS sequences of genus *Spiraea* were conducted using the Nei-Gojobori method.

A pairwise alignment was calculated using the Clustal X program. Phylogenetic relationship were estimated by MEGA version 5 [16] treating all alignment gaps as missing. Confidence values for individual branches were determined by a bootstrap analysis with 1000 repeated sampling of the data.

Maximum parsimony: Heuristic searches were implemented in MEGA version 5 [16,17]under the maximum parsimony (MP) criteria with the accelerated transformation (ACCTRAN) option to optimize the state of unordered (Fitch) characters, 100 random sequence addition replicates, the tree bisection-reconnection (TBR) branch swapping, and gaps treated as a fifth character. Unweighted MP methods do not always take full advantage of the information contained in DNA sequences due to the presence of homoplasious characters. To deal with this problem, a weighted parsimony analysis was also conducted using the rescaled consistency index on an initial tree in successive approximations of character weighting [1].

Maximum likelihood: Themaximum-likelihood (ML) search was conducted using PAUP*4.0b10 [15]. Namely, I first evaluated the model of DNA substitution that best fit my data partition, using the Akaike information criterion in MODELTEST 3.0 [10]. Then we calculated the ML model parameters using the data partition of interest and a neighbor-joining topology. These estimated values were fixed, and a full heuristic ML search was conducted with 10 random addition sequence replicates, retaining all minimal trees, and TBR branch swapping. After this first ML search was completed, I reestimated model parameters on the ML tree and used these new values to search again. These processes were repeated until the new search found the same topology as the previous search and all parameter estimates were identical.

Results

ITS region for fourteen taxa of *Spireae* in Korea was successful in all of the species. Aligned nucleotide sequences of ITS were varied within *Spireae* varying from 625 in *S. pubescens* to 656 in *S. trichocarpa*.

Total alignment length is 689 positions, of which 109 are parsimony-informative, 249 variable, 138 singleton, and 412 constant characters. The base furtherance did not showed a significant difference to the by a total taxa (Table 2). The mean nucleotide frequencies for fourteen taxa of genus *Spireae* in Korea are A=16.9%, C=30.8%, G=34.3%, and T=18.0% (Table 2).

All ITS trees generated in Korea exhibited well solved topology with high bootstrap support irrespective of the methods (parsimony) and the setting used. This result confirmed monophyletic group for all species (Fig. 1). *P. pubescens* was more distinct in ML and MP trees. *S. chamaedryfolia* var. *ulmifolia* was strongly supported with a bootstrap value of 96% and *S. fritschiana* had also strongly supported with a bootstrap (100%). The close relationships of *S. prunifolia* for. *simpliciflora* and *S. blumei*, *S. chartacea* and *S. cantoniensis*, and *S. fritschiana* var. *obtusifolia* and *S. miyabei* were supported with high bootstrap values (100%, 99%, and 95%, respectively).

In 47 world accessions (38 taxa: 14 Korean taxa, 33 world taxa, and 9 overlapped same taxa), total alignment length is 689 positions, of which 452 are parsimony-informative, 527 variable, 75 singleton, and 159 constant characters.

Substitution pattern and rates were estimated under the Kimura 2-parameter model. The estimated Transition/ Transversion biases (R) varied from 0.50 to 0.96. Under maximum likelihood fits of 24 different nucleotide substitution models, Substitution from A to G was 22.5 and the reverse was 10.73 (Table 3).

BIC score was the lowest at the Kimura parameter with 4959.9 (Table 4). AICc value was the lowest at the Tamura 3-parameter with 4762.3. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well.

Number of segregating sites was 430 and nucleotide diversity (π) was 0.281. Under the neutral mutation hypothesis, the probability that the Tajima test statistic (D) is positive (2.325) is more than 0.5 (Table 5). Therefore, there may be

Table 2. List of genus *Spireae* from GenBank accession numbers

Taxa	Distribution	Authors	No. of GenBank
S. blumei G. Don.	China, Japan, Korea	Potter et al. [11]	DQ897607
S. canescens D. Don.	Himalaya	Potter et al. [11]	DQ897608
S. cantoniensis Lour.	China, Japan, Korea	Potter et al. [11]	DQ897609
S. chamaedryfolia L.	China, Korea	Oh et al. [9]	GU217795
S. crenata L.	Europe, Asia	Potter et al. [11]	DQ897610
S. decumbens W. Koch.	Europe	Potter et al. [11]	DQ897611
S. densiflora Nutt.	U.S.A	Potter et al. [11]	DQ886362
S. douglasii Hook.	British Columbia, Canada	Potter et al. [11]	DQ897612
S. formosana Hayata	Taiwan	Potter et al. [11]	DQ897613
S. fritschiana Schneid.	China, Korea	Potter et al. [11]	DQ897614
S. hypericifolia L.	Europe, Asia	Potter et al. [11]	DQ897615
S. japonica L.	Japan	Potter et al. [11]	DQ897616
S. japonica var. *acuta* Yu	China	Zhang et al. [21]	AY742242
S. japonica var. *acuminata* Franch	China	Zhang et al. [21]	AY742239
S. japonica var. *fortune* Schneid.	China	Zhang et al. [21]	AY742232
S. japonica var. *glabra* Koidz.	China	Zhang et al. [21]	AY74223
S. japonica var. *japonica* Schneid.	Japan	Zhang et al. [21]	AY742231
S. japonica var. *incise* Yu	China	Zhang et al. [21]	AY742241
S. japonica var. *ovalifolia* Franch	China	Zhang et al. [21]	AY742235
S. japonica var. *stellaris* Rehrd.	China	Zhang et al. [21]	AY742238
S. lasiocarpa Kar. & Kir.	Russia	Potter et al. [11]	DQ897618
S. latifolia (Ait.) Borkh.	Canada, U.S.A.	Potter et al. [11]	DQ897619
S. longigemmis Maxim.	China	Potter et al. [11]	DQ897620
S. miyabei Koidz.	China	Potter et al. [11]	DQ897621
S. nipponica Maxim.	Japan	Potter et al. [11]	DQ897622
S. prunifolia Sieb. & Zucc.	China, Korea, Taiwan	Potter et al. [11]	DQ897623
S. pubescens Turcz.	China, Korea	Potter et al. [11]	DQ897624
S. salicifolia L.	Europe, Asia	Potter et al. [11]	DQ897625
S. thunbergii Sieb.	Japan, Korea	Potter et al. [11]	DQ897626
S. trichocarpa Nakai	Korea	Potter et al. [11]	DQ897627
S. trilobata L.	Asia	Potter et al. [11]	DQ897628
S. veitchii Hemsl.	China	Potter et al. [11]	DQ897629
S. virginiana Brit.	U.S.A.	Potter et al. [11]	DQ897631

Table 3. Base frequencies across fourteen taxa of genus *Spireae* in Korea using ITS analysis

Taxa	T	C	A	G	Total
S. chamaedryfolia var. *ulmifolia*	20.1	29.8	17.2	33.0	652.0
S. chartacea	17.0	30.8	16.7	35.4	652.0
S. chinensis	17.8	31.2	16.5	34.6	642.0
S. fritschiana var. *obtusifolia*	17.8	31.2	16.4	34.7	642.0
S. fritschiana	20.2	28.8	18.0	33.0	643.0
S. japonica	17.8	31.3	16.4	34.6	642.0
S. miyabei	17.8	31.2	16.4	34.7	642.0
S. prunifolia for. *simpliciflora*	17.8	31.0	16.9	34.4	652.0
S. pubescens	18.7	31.7	18.9	30.7	625.0
S. salicifolia	17.2	30.9	15.3	36.6	653.0
S. trichocarpa	17.4	31.4	17.4	33.8	656.0
S. blumei	17.7	31.0	16.9	34.4	651.0
S. cantoniensis	17.5	30.3	17.2	35.1	653.0
S. thunbergii	17.8	30.8	16.7	34.7	652.0
Avg.	18.0	30.8	16.9	34.3	646.9

Table 4. Maximum likelihood estimate of the pattern of nucleotide sequences

Base	A	T	C	G
A	-	*4.33*	*7.41*	**22.5**
T	*3.94*	-	**11.92**	*8.27*
C	*3.94*	**6.96**	-	*8.27*
G	**10.73**	*4.33*	*7.41*	-

Each entry shows the probability of substitution (r) from one base (row) to another base (column). For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in *italics*. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 689 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

a site at which natural selection, which increases the genetic variation, is operating.

Phylogenetic reconstructions revealed notable differences in world wide taxa (Figs. 2 and 3), indicating a more or less constant evolution rate in the genus. Many clades of same taxa were not supported and did not agree with the topology positions for the same scientific taxa. The outgroup was omitted because of length of tree.

S. blumei, *S. cantoniensis*, *S. fritschiana*, *S. japonica*, *S. miyabei*, *S. pubescens*, *S. salicifolia*, *S. thunbergii*, and *S. trichocarpa* occupy Korea and other Asian countries, Europe, and North America. They are not clustered with each other (Figs. 2 and 3). The eight *S. japonica* varieties did not formed a clade. In addition, *S. japonica* varieties were not also sistered with

Table 5. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Param	BIC	AICc	lnL	Invariant	Gamma	R
T92+G	28	4959.9	4762.3	-2353.1	n/a	0.37786	1.191
T92+G+I	29	4968.8	4764.2	-2353	0.128085	0.48605	1.1918
T92+I	28	4974.6	4777	-2360.4	0.521695	n/a	1.1575
HKY+G	30	4979.3	4767.7	-2353.7	n/a	0.38209	1.1835
TN93+G	31	4982.5	4763.8	-2350.8	n/a	0.40022	1.1825
HKY+G+I	31	4988.2	4769.5	-2353.6	0.128723	0.49289	1.1843
TN93+G+I	32	4991.4	4765.6	-2350.7	0.128069	0.51774	1.1836
HKY+I	30	4993.6	4782	-2360.9	0.520596	n/a	1.151
TN93+I	31	4997.1	4778.4	-2358.1	0.510795	n/a	1.1446
GTR+G	34	5000.6	4760.8	-2346.2	n/a	0.3987	1.1986
GTR+G+I	35	5009.4	4762.5	-2346.1	0.148812	0.54301	1.1999
GTR+I	34	5014.7	4774.8	-2353.3	0.504706	n/a	1.1509
K2+G	27	5022.6	4832.1	-2388.9	n/a	0.37761	1.1652
K2+G+I	28	5031.3	4833.8	-2388.8	0.144248	0.5005	1.1669
K2+I	27	5038.5	4848	-2396.9	0.528449	n/a	1.1393
JC+G	26	5051.2	4867.7	-2407.8	n/a	0.37847	0.5
JC+G+I	27	5059.9	4869.4	-2407.6	0.149576	0.50588	0.5
T92	27	5062.2	4871.7	-2408.7	n/a	n/a	1.0276
TN93	30	5078.7	4867	-2403.4	n/a	n/a	1.0368
HKY	29	5080.8	4876.2	-2409	n/a	n/a	1.0282
GTR	33	5097.2	4864.4	-2399.1	n/a	n/a	1.0345
K2	26	5124.5	4941.1	-2444.4	n/a	n/a	1.0264
JC	25	5151.1	4974.7	-2462.3	n/a	n/a	0.5
JC+I	26	5160.2	4976.7	-2462.3	0.00001	n/a	0.5

T92: Tamura 3-parameter; G: Gamma distribution, I: Evolutionarily invariable, GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; K2: Kimura 2-parameter; JC: Jukes-Cantor.

Table 6. Results from Tajima's neutrality test for ITS sequences of genus *Spireae*

Group	M	S	ps	Θ	Π	D
Korea taxa	14	201	0.337	0.106	0.088	-0.737
Foreign taxa	33	73	0.135	0.033	0.030	-0.343
Total	47	382	0.759	0.172	0.281	2.325

M=number of sites, S=Number of segregating sites, ps=S/M, Θ=ps/a1, and π=nucleotide diversity. D is the Tajima test statistic.

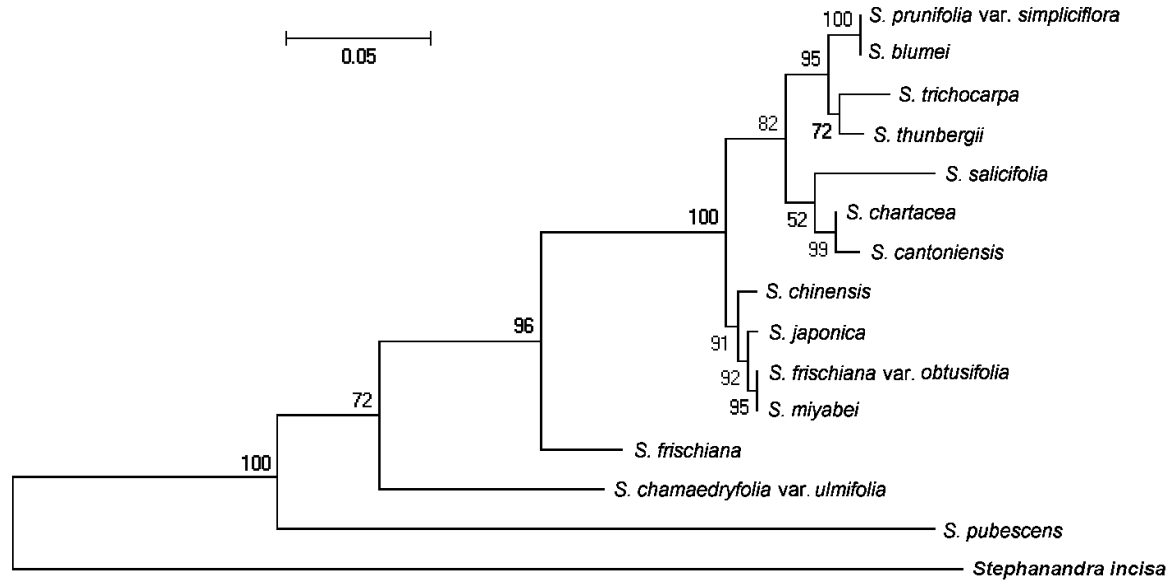

Fig. 1. The maximum likelihood tree for Korean taxa of genus *Spireae* based on ITS analysis using MEGA5. The values of bootstrap were shown in side of vertical lines.

S. japonica.

Discussion

The internal structure of the genus *Spireae* is by and large in discordances with other recent and more densely sampled DNA phylogenies of the *Spiraeea*, such as those of Porter et al. [11]. The sparse sampling of *Spireae* taxa in the present study, together with a relatively high DNA sequence mutation rate, made alignment of *Spireae* sequences a difficult task, and because of this difficulty, an insertion/deletion complex unique to the one *Spireae* taxa had to be removed from the data matrix before analysis.

Although all taxa in Korea exhibited well solved topology with high bootstrap, phylogenetic reconstructions revealed notable differences in world wide taxa (Figs. 2 and 3). This discordance in *Spireae* tree may be explained with two possible explanations. First, the accurate identification of *Spireae* species has always been problematic even for expert botanists. This is because of the contradictory classification systems proposed by various researchers, primarily based on morphological characters that could be highly variable depending on the environmental conditions. So, it cannot rule out that the some taxa are not simple species or pure accessions. For example, *S. japonica* var. *glabra* Koidz (AY74223) and *S. japonica* var. *acuta* Yu (AY742242) are the varieties of *S. japonica*. However, var. *glabra* is distinct from other varieties of *S. japonica* (see sequences and Figs. 2 and 3).Second, amplification regions of ITS are different from the methods and primers by authors. Although pairwise alignments were calculated using the Clustal X program, the alignments could be had the limitation of accuracy. For example, *S. fritschiana* (DQ897614) is very similar to many taxa such as S. miyabei (DQ897621), *S. japonica* var. *fortunei* (AY742232), *S. japonica* (DQ897616), *S. japonica* var. *glabra* (AY742130), *S. japonica* var. *glabra* (AY742132), *S. formosana* (DQ897613), and so on.

Inflorescence type, the basis for the recognition of three sections within Spiraea [13] does not appear to be a reliable indicator of relationship within the genus (Figs. 2 and 3), but some correlations were nonetheless observed. While compound corymbs, characteristic of section *Calospira*, are found in *S. fritschiana* var. *obtusifolia*, *S. fritschiana*, and *S. miyabei*, here resolved as sister to section *Chamaedryon* and restricted to one strongly supported clades and panicles (section *Spiraea*) are found in one clade (strongly supported by Bayesian analysis) in some of the most parsimonious trees.

The plants of genus *Spireae* grow in forest-steppe, steppe, semi-dessert zones, and in subalpine belt of the mountains of the North Hemisphere. This environmental heterogeneity could have resulted in more geographical barriers, higher

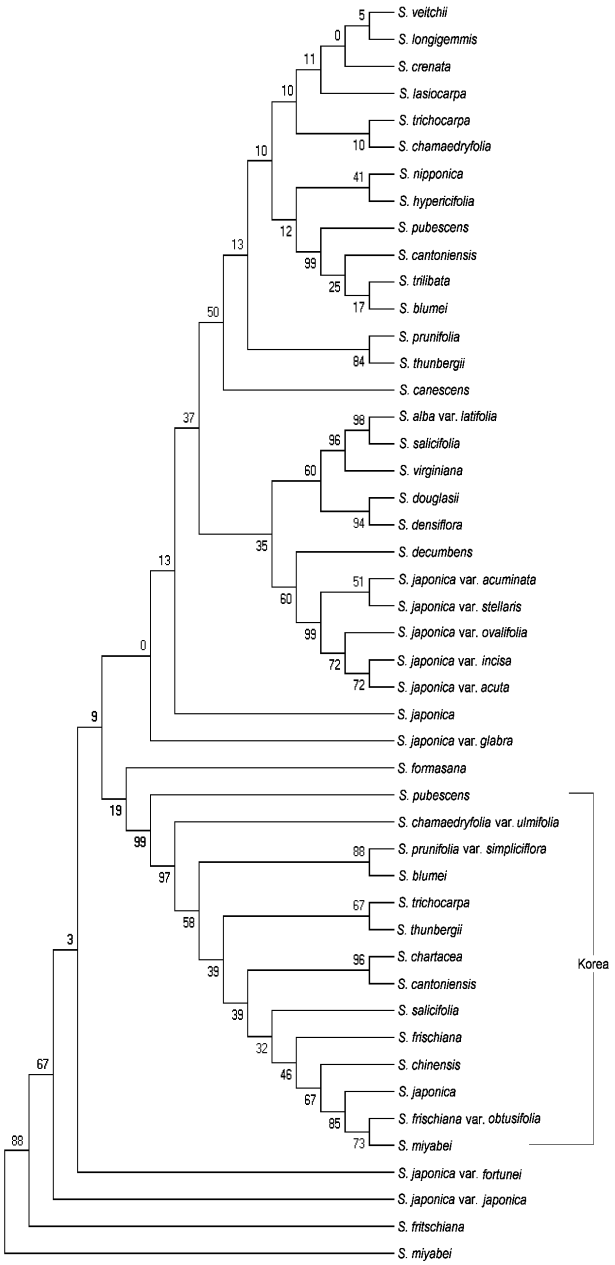

Fig. 2. The maximum parsimonious tree for genus *Spireae* based on ITS using PAUP 4b10, exhaustive search, unweighted parsimony analysis, gaps=fifth state) from the 689 aligned positions of the initial matrix. The values of bootstrap were shown in side of vertical lines.

Fig. 3. The maximum likelihood tree for genus *Spireae* based on ITS analysis using MEGA5. The values of bootstrap were shown in side of vertical lines.

selection pressures, greater isolation, and less gene flow between populations. All these factors might explain the higher divergence and diversification among *S. japonica* varieties. It is a general principle that the higher the genetic divergence among phylogenetic lines, the earlier the many taxa diverged. This has been widely applied in the interpretation of biogeographical history using sequence data [14]. A possible explanation might be that different areas with different environments will vary in mutation accumulation or divergence maintenance and consequently differ in detectable evolutionary rate.

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초록 : 핵 리보솜 DNA ITS 부위에 의한 조팝나무속 식물종의 계통 관계 분석

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조팝나무속(genus *Spiraea*) 식물은 다년생 목본으로 주로 아시아와 유럽에 분포하고 있다. 한국의 14종을 포함한 전 세계 38분류군에 대해 핵 내 리보솜 전사 서열(ITS)로 이 속의 유전적 관계를 평가하였다. 이 분자생물학적 자료로 분류군의 분지군은 잘 분리되었다. 47 계통(38 분류군: 14개 한국 분류군, 33개 세계 분류군, 9개 중복 분류군). 전체 689 bp 중에서452자리는 절약-정보적이었고, 527자리는 변이를 나타내었으나 절약-비정보적이었고, 159자리는 분류군 전체에서 변이가 전혀 없었다. 비록 계통도에서 잘 분리되었지만 형태적 특성과 지리적 분포와는 일치하지 않았다. 분리되는 자리수는 430이었으며 핵산 다양도(π)는 0.281이였다. 중립가설 하에서 Tajima 검증 통계값(D) 은 0.5보다 큰 2.325였다. 따라서 자연 도태가 유전적 변이를 증가시키는 방향으로 작용하고 있었다.