Taxonomic study on infraspecific taxa of Lespedeza maximowiczii and hybrids with related species

Dong-Pil JIN, Jong-Won PARK and Byoung-Hee CHOI*

Department of Biological Sciences, Inha University, Incheon 22212, Korea

(Received 12 November 2019; Revised 14 December 2019; Accepted 25 December 2019)

ABSTRACT: Many infraspecific taxa within Lespedeza maximowiczii and hybrids with related species have been described, but taxonomic verification remains controversial. We examined the morphological traits of hybrids (L. chisanensis and L. patentibicolor) and infraspecific taxa (var. tomentella, elongata, and tricolor) and analyzed their genetic structures using microsatellite loci. Flower and leaflet shapes in var. tomentella and elongata were within the range of variation of those in var. maximowiczii, and individuals in the two former varieties were grouped into var. maximowiczii. Lespedeza maximowiczii var. tricolor was similar to L. buergeri in terms of the structure and flower color, whereas the leaflet and bracteole shapes of var. tricolor were similar to those of var. maximowiczii. Based on the genetic structure (K = 3), var. tricolor had a mixed lineage with L. maximowiczii and L. buergeri. In addition, these formed a distinct lineage at K = 5. For two hybrids, the flower and leaflet structure in L. chisanensis did not differ from those in L. maximowiczii, whereas the flowers of L. patentibicolor were within the range of variation of L. bicolor. In addition, L. chisanensis and L. patentibicolor were assigned to L. maximowiczii and L. bicolor, respectively, based on the genetic structure. We treated var. tomentella and elongata as a forma, f. friebeana, because L. friebeana preceded var. tomentella, whereas var. tricolor was treated as a distinct species, L. tricolor. Lespedeza chisanensis was recognized as a synonym of L. maximowiczii. Lespedeza patentibicolor was considered to be L. bicolor.

Keywords: Hybrid, infraspecific taxa, Lespedeza maximowiczii, morphological variation, microsatellites, taxonomic treatment

The genus Lespedeza Michx., which belongs to the family Fabaceae, includes approximately 44 species and is disjunctively distributed in Asia and North America (Ohashi and Nemoto, 2014). Within this genus, two subgenera [Lespedeza and Macrolespedeza (Maxim.) H. Ohashi] were recently circumscribed based on the morphology of seedlings and molecular data (Ohashi and Nemoto, 2014). The former grows in the eastern region of North America, whereas the latter is distributed from East Asia to India. In the subgenus Macrolespedeza, the section Macrolespedeza is composed of subshrub and shrub that bears only chasmogamous flowers, whereas the section Junceae (Maxim.) H. Ohashi & T. Nemoto is composed of herbs and subshrubs that bear both chasmogamous and cleistogamous flowers (Ohashi and Nemoto, 2014). The members of the section Macrolespedeza are morphologically varied (Lee, 1965; Akiyama, 1988), which hinders the identification of some species. Many hybrids in the taxa of this section have been reported based on morphological evidence (Lee, 1965; Akiyama, 1988, 2004). Previous molecular phylogenies showed the signal for hybridization within this section (Xu et al., 2012, 2017), i.e., most species are not supported as monophyletic according to their taxonomic status.

Thirteen Lespedeza species have been recognized in Korea and six belong to the section Macrolespedeza (Choi, 2007). In this study, we focused on L. maximowiczii C. K. Schneid., which is in the section. Until recently, this species was known to be distributed in China, Korea, and Tsushima Island of Japan (Ohashi et al., 2009). However, the Chinese plant was separated into L. pseudomaximowiczii D. P. Jin, B. Xu & B.
H. Choi because of its distinct morphology and genetic features (Jin et al., 2018); thus, it is accepted that *L. maximowiczii* grows in Korea and a narrow area of Tsushima Island. Like other *Macrolespedeza* species, many infraspecific taxa of *L. maximowiczii* have been recorded, including var. *elongata* Nakai, var. *tomentella* Nakai, var. *tricolor* Nakai, and f. *albiflora* Uyeki (Nakai, 1927; Uyeki, 1941). The taxonomic ranks of these taxa have been argued by different researchers (Hatusima, 1967; Akiyama, 1988). For example, Hatusima (1967) treated *L. maximowiczii* as a subspecies [= subsp. *praecox* (Nakai) Hatusima] of closely related species, *L. buergeri* Miq., var. *tomentella*, and var. *tricolor* were simultaneously changed to the forma [= f. *tomentella* (Nakai) Hatusima] of subsp. *praecox* and other subspecies [= subsp. *tricolor* (Nakai) Hatusima], respectively. However, Akiyama (1988) treated var. *tomentella* as a synonym of *L. maximowiczii*. Furthermore, *L. maximowiczii* var. *elongata* and var. *tricolor* were thought to be synonyms of *L. maximowiczii* (Akiyama, 1988). Many hybrids related to *L. maximowiczii* have been recognized in Korea (Lee, 1965). In detail, the following hybrids were described: *L. angustifolioides* T. Lee (including *L. maximowiczii* var. *elongata* as its synonym) [i.e., *L. maximowiczii* × *L. japonica* L. H. Bailey var. *intermedia* (Nakai) Nakai], *L. chisamensis* T. Lee (*L. maximowiczii* × *L. bicolour* Trucz.), *L. maritima* Nakai (*L. maximowiczii* × *L. cyrbotrya* Miq.), *L. patentiibicolor* T. Lee (*L. maximowiczii* var. *tomentella* × *L. bicolour*), and *L. patentielongata* T. Lee (*L. maximowiczii* var. *tomentella* × *L. japonica* var. *intermedia*). In Lee’s subsequent study, he regarded *L. maximowiczii* var. *tomentella* as a hybrid species, i.e., *L. × tomentella* (Lee, 1980) [= *L. × friebenea* Schindl. in Lee and Lee (1975)], between *L. maximowiczii* and *L. japonica* var. *intermedia* [= *L. thunbergii* (DC.) Nakai subsp. *thunbergii* in Ohashi et al. (2009)]. Furthermore, two hybrids (*L. angustifolioides* and *L. patentielongata*) and *L. maximowiczii* var. *elongata* were circumscribed into *L. × friebenea* in that paper (Lee and Lee, 1975). In the case of *L. patentiibicolor*, because one putative parent was treated as a hybrid (Lee and Lee, 1975), this species would be a hybrid between a species (*L. maximowiczii*) and a hybrid (*L. × friebenea*). Although Lee’s studies improved our understanding of the Korean *Lespedeza* species (Lee, 1965; Lee and Lee, 1975), flowers have not been sufficiently examined despite their importance for identification (Akiyama, 1988). Genetic evidence regarding hybrids is also lacking. Therefore, resolving this complex taxonomic issue related to *L. maximowiczii* requires morphological and genetic analyses.

To verify hybridization, a putative hybrid needs to be examined by genetic evidence and morphological characters. Partial chloroplast DNA and nuclear ribosomal internal transcribed spacer (nrITS) data do not appropriately delimit the species (Xu et al., 2012, 2017), perhaps because of their frequent hybridization or incomplete lineage sorting. Thus, we applied microsatellite markers developed from *L. maritima* (Jin et al., 2016a) to these taxa. Microsatellite markers are codominant and highly polymorphic. Furthermore, they may cover multiple loci in the nuclear DNA (Duminil et al., 2012). Microsatellite markers show the hybridization and introgression between species, in genera such as *Quercus* L. (Lee et al., 2014; Castillo-Mendoza et al., 2019), *Ulmus* L. (Brunet et al., 2013), and *Populus* L. (Zeng et al., 2016). The genetic traits of *L. pseudomaximowiczii* were also revealed by the comparison with other *Lespedeza* species using microsatellite markers (Jin et al., 2018).

Here, our goals were to (1) examine the morphological characters of infraspecific taxa of *L. maximowiczii* and hybrids with related species, (2) investigate the genetic structure among taxa using microsatellite loci, and (3) discuss the taxonomic entities based on these results.

### Materials and Methods

**Morphological examination and survey of geographical distribution**

To compare the morphological characters of typical *L. maximowiczii* with infraspecific taxa and hybrids, we collected specimens from the field sampling and deposited them in the Herbarium of Inha University (IUI). The sample information is detailed in Table 1. Additional morphological examination was conducted by observing specimens from the Korea National Arboretum (KH), College of Agriculture Life Sciences, Seoul National University (SNUA), Institute of Botany, Chinese Academy of Sciences (PE), and the Herbarium of University of Tokyo (TI). In particular, we observed T. B. Lee’s collections to understand how he regarded hybrids in his previous studies (Lee, 1965), i.e., *L. chisamensis*, *L. patentiibicolor*, *L. angustifolioides*, and *L. patentielongata* (Fig. 1). Among these, the latter two were regarded as a hybrid of *L. maximowiczii* and *L. thunbergii* (*L. × friebenea* [= *L. × tomentella*]), including *L. maximowiczii* var. *elongata* (Lee and Lee, 1975). *L. angustifolioides*, *L. patentielongata*, and *L. maximowiczii* var. *elongata* were characterized by acuminate at both ends of the leaflet. The two former were distinguished based only on the hairs on the inflorescence (appressed vs.
Sampling, DNA extraction, and the microsatellite polymerase chain reaction (PCR)

Leaves were sampled from the voucher specimens shown in Table 1 and preserved using silica gel. The dried leaves from *Lespedeza* species were used for genomic DNA extraction. We used an MG Plant Genomic DNA Extraction SV Miniprep Kit (MGmed, Seoul, Korea). Although we mostly followed the manufacturer’s instructions, initial incubation and ice incubation times were extended to acquire sufficient DNA.

We used the following 11 microsatellite loci (Jin et al., 2016a): LMS3, LMS7, LMS11, LMS18, LMS28, LMS33, LMS39, LMS45, LMS47, LMS53, LMS55, and LMS61. The PCR protocol utilized these markers with a GeneAmp PCR System 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Each reaction mixture (10 μL total volume) contained 5 ng of DNA, plus 5 μL of 2× Plus Mix (Dongsheng Biotech, Guangdong, China) that comprised 0.4 mM dNTPs, 2× PCR buffer with 4 mM MgSO$_4$, and 0.4 U/μL of Taq DNA polymerase. The mixtures also contained the appropriate primer, and a 0.3 μM M13 (-21) labeled fluorescent marker. The PCR protocol utilized these markers with a GeneAmp PCR System 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Each reaction mixture (10 μL total volume) contained 5 ng of DNA, plus 5 μL of 2× Plus Mix (Dongsheng Biotech, Guangdong, China) that comprised 0.4 mM dNTPs, 2× PCR buffer with 4 mM MgSO$_4$, and 0.4 U/μL of Taq DNA polymerase. The mixtures also contained the appropriate primer, and a 0.3 μM M13 (-21) labeled fluorescent marker.

The PCR products were visualized on 2% agarose gels and resolved to genotype on an ABI 3730XL sequencer with GeneScan 500 LIZ size standards (Applied Biosystems). This fragment analysis was performed by Macrogen, Inc. (Seoul, Korea). The sizes of the alleles were manually determined using the program GENEMAPPER 3.7 (Applied Biosystems).

---

Table 1. Sampling information for *Lespedeza maximowiczii* and its related species in Korea that were used in this study.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Code</th>
<th>Population locality</th>
<th>Coordinates</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. maximowiczii</em> f. maximowiczii (조록싸리)</td>
<td>JR</td>
<td>Jirisan Mt., Namwon, Jeonbuk, Korea</td>
<td>35°17'N, 127°31'E</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>Deoksung Mt., Yesan, Chungnam, Korea</td>
<td>36°40'N, 126°37'E</td>
<td>8</td>
</tr>
<tr>
<td><em>L. maximowiczii</em> var. tomentella (= f. friebeanu) (털조록싸리)</td>
<td>CC</td>
<td>Cheomchalsan Mt., Uisin, Jeondo, Jeonnam, Korea</td>
<td>34°28'N, 126°19'E</td>
<td>9</td>
</tr>
<tr>
<td><em>L. maximowiczii</em> var. elongata (= f. friebeanu) ( awakeFromNib</td>
<td>JR</td>
<td>Jirisan Mt., Namwon, Jeonbuk, Korea</td>
<td>35°17'N, 127°31'E</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>Deoksung San Mt., Yesan, Chungnam, Korea</td>
<td>36°40'N, 126°37'E</td>
<td>8</td>
</tr>
<tr>
<td><em>L. maximowiczii</em> var. tricolor (= L. tricolor) (삼색싸리)</td>
<td>CC</td>
<td>Cheomchalsan Mt., Uisin, Jeondo, Jeonnam, Korea</td>
<td>34°28'N, 126°19'E</td>
<td>10</td>
</tr>
<tr>
<td><em>L. patentibicolor</em> (간도싸리)</td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td><em>L. chisanensis</em> (= <em>L. maximowiczii</em> f. maximowiczii) (지리산싸리)</td>
<td>JR</td>
<td>Jirisan Mt., Namwon, Jeonbuk, Korea</td>
<td>35°17'N, 127°31'E</td>
<td>2</td>
</tr>
<tr>
<td><em>L. bicolor</em> (바리)</td>
<td>DY</td>
<td>Hyangsan 7-gil, Danyang, Chungbuk, Korea</td>
<td>37°03'N, 128°26'E</td>
<td>7</td>
</tr>
<tr>
<td><em>L. buergeri</em></td>
<td>TB</td>
<td>Taihaisan Mt., Lushi, Henan, China</td>
<td>33°52'N, 111°19'E</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>Kayagasan Mt., Hokuto, Yamanashi, Japan</td>
<td>35°47'N, 138°30'E</td>
<td>15</td>
</tr>
<tr>
<td><em>L. thunbergii</em> subsp. thunbergii (동바리)</td>
<td>BS</td>
<td>Jangsangbongsan Mt., Busan, Korea</td>
<td>35°6'N, 129°6'E</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>Cheomchalsan Mt., Uisin, Jeondo, Jeonnam, Korea</td>
<td>34°28'N, 126°19'E</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>Deoksung San Mt., Yesan, Chungnam, Korea</td>
<td>36°40'N, 126°37'E</td>
<td>3</td>
</tr>
</tbody>
</table>

patent) (Lee, 1965). Thus, we considered them to be *L. maximowiczii* var. elongata. Additional specimen examination was conducted using the website of the Royal Botanical Garden Kew (K), Naturalis (L), and Global Biodiversity Information Facility (GBIF) dataset published by the T. B. Lee herbarium (Chang and Kim, 2019). We could not observe specimens of *L. maximowiczii* f. albiflora; therefore, this taxon was not included in this study. All observations of morphological characters were made with a stereomicroscope (Leica MZ8; Wetzlar, Germany), and floral features were measured following criteria that were stipulated by Akiyama (1988).
Fig. 1. Four *Lespedeza* hybrids that were investigated in this study. These were recognized by Lee (1965, 1980). A. *L. chisanensis* (lectotype). B. *L. patentibicolor* (holotype). C. *L. angustifolioides* (lectotype). D. *L. patentielongata* (holotype).
Microsatellite data analyses

The fragment sizes of all loci were genotyped according to the individual. To determine the assignment pattern of the six taxa, Bayesian clustering was conducted using our genotypic data. This analysis was performed with STRUCTURE 2.3.4 (Pritchard et al., 2000), selecting the admixture ancestry and correlated allele frequency models. Ten iterations were run for each cluster (K = 1 to 20), with 30,000 burn-ins followed by 300,000 Markov chain Monte Carlo repetitions. The optimum K value was determined based on ΔK according to the number of clusters, which was estimated using STRUCTURE HARVESTER (Earl and von Holdt, 2012). The results from each run were generated to a representative stacked bar chart using the Clustering Markov Packager Across K (CLUMPAK) on the web (Kopelman et al., 2015) to summarize and generate representative pie charts associated with each K value. To represent the relationship among species, we reconstructed the neighbor-joining (NJ) tree based on Nei’s genetic distances (D_A) (Nei et al., 1983). D_A between individuals was calculated with the POPULATIONS 1.2.32 (Langella, 2011).

Results

Morphological examination on infraspecific taxa of L. maximowiczii and hybrids with related species

We compared morphological characters to reconsider the taxonomic position of taxa related to L. maximowiczii. The measured values of the infraspecific taxa and hybrids are shown in Tables 2 and 3, respectively.

The results for the infraspecific taxa (Fig. 2, Table 2) showed that L. maximowiczii var. tomentella (8.9–[10.1]–11.2 mm) had slightly longer flowers than those of var. elongata (9.0–[9.6]–10.2 mm) and var. maximowiczii (8.2–[9.5]–10.9 mm). Flowers of var. tricolor (8.4–[9.0]–9.5 mm) were slightly shorter than those of var. maximowiczii, which were similar to those of L. buergeri (8.2–[9.0]–9.7 mm). The color of standard petals (purple) was almost identical among the infraspecific taxa, whereas var. tricolor had a pale-yellow standard petal with purple dots inside, which was similar to that of L. buergeri. The order of the length of petals [K (keel petal) ≒ S (standard petal) > W (wing petal)] was the same in var. maximowiczii

<table>
<thead>
<tr>
<th>L. maximowiczii var. maximowiczii</th>
<th>var. tomentella (= f. friebeana)</th>
<th>var. elongata (= f. friebeana)</th>
<th>var. tricolor (= L. tricolor)</th>
<th>L. buergeri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair on inflorescence</td>
<td>Adpressed to patent hair</td>
<td>Adpressed to patent hair</td>
<td>Adpressed hair</td>
<td>Adpressed hair</td>
</tr>
<tr>
<td>Length of flower (mm)</td>
<td>8.2–(9.5)–10.9</td>
<td>8.9–(10.1)–11.2</td>
<td>9.0–(9.6)–10.2</td>
<td>8.4–(9.0)–9.5</td>
</tr>
<tr>
<td>Color of standard petal</td>
<td>Purple with denser purple dot inside</td>
<td>Purple with denser purple dot inside</td>
<td>Purple with denser purple dot inside</td>
<td>Pale yellow with purple dot inside</td>
</tr>
<tr>
<td>Length of standard petal (mm)</td>
<td>7.0–(8.5)–10.0</td>
<td>8.2–(9.3)–10.4</td>
<td>8.0–(8.9)–9.6</td>
<td>6.7–(7.6)–8.6</td>
</tr>
<tr>
<td>Length of standard petal claw (mm)</td>
<td>0.7–(1.1)–1.5</td>
<td>0.8–(1.2)–1.5</td>
<td>0.8–(1.1)–1.4</td>
<td>1.0–(1.2)–1.7</td>
</tr>
<tr>
<td>Length of wing petal (mm)</td>
<td>6.4–(7.5)–9.2</td>
<td>7.1–(8.6)–9.9</td>
<td>7.4–(8.3)–9.0</td>
<td>6.5–(7.4)–8.3</td>
</tr>
<tr>
<td>Length of wing petal claw (mm)</td>
<td>1.7–(2.4)–2.9</td>
<td>1.6–(2.4)–2.9</td>
<td>2.1–(2.5)–2.9</td>
<td>2.0–(2.5)–2.9</td>
</tr>
<tr>
<td>Length of wing petal lamina (mm)</td>
<td>4.5–(5.4)–6.7</td>
<td>4.8–(6.3)–7.3</td>
<td>5.0–(6.0)–6.8</td>
<td>4.5–(5.0)–5.6</td>
</tr>
<tr>
<td>Length of keel petal (mm)</td>
<td>7.8–(8.8)–9.8</td>
<td>8.8–(9.5)–10.7</td>
<td>8.2–(9.0)–9.7</td>
<td>8.3–(8.7)–9.0</td>
</tr>
<tr>
<td>Length of keel petal claw (mm)</td>
<td>2.0–(2.6)–3.3</td>
<td>2.4–(2.8)–3.3</td>
<td>2.3–(2.6)–2.8</td>
<td>2.1–(2.6)–2.9</td>
</tr>
<tr>
<td>Length of keel petal lamina (mm)</td>
<td>5.6–(6.3)–7.0</td>
<td>6.2–(6.9)–7.5</td>
<td>6.1–(6.6)–7.0</td>
<td>5.4–(6.1)–6.4</td>
</tr>
<tr>
<td>Length of calyx tube (mm)</td>
<td>1.2–(1.5)–2.1</td>
<td>1.4–(1.8)–2.2</td>
<td>1.2–(1.4)–1.6</td>
<td>1.3–(1.5)–1.6</td>
</tr>
<tr>
<td>Length of calyx lobe (mm)</td>
<td>1.5–(2.3)–3.5</td>
<td>1.8–(3.1)–4.1</td>
<td>2.5–(3.0)–3.2</td>
<td>0.7–(0.9)–1.1</td>
</tr>
<tr>
<td>Length of bracteoles (mm)</td>
<td>0.5–(1.1)–2.1</td>
<td>0.4–(1.0)–1.9</td>
<td>1.2–(1.5)–1.8</td>
<td>0.8–(1.1)–1.4</td>
</tr>
<tr>
<td>Width of bracteoles (mm)</td>
<td>0.2–(0.5)–1.0</td>
<td>0.2–(0.4)–0.6</td>
<td>0.3–(0.4)–0.6</td>
<td>0.6–(0.7)–0.8</td>
</tr>
<tr>
<td>Shape of leaflets at apex</td>
<td>Mostly acute to acuminate</td>
<td>Mostly acute to acuminate</td>
<td>Acuminate</td>
<td>Mostly acute to acuminate</td>
</tr>
<tr>
<td>Acute to obtuse</td>
<td></td>
<td></td>
<td></td>
<td>Acute to obtuse</td>
</tr>
<tr>
<td>Density of hairs on upper surface of leaflet (number per 4 mm²)</td>
<td>1–(8)–59</td>
<td>38–(51)–90</td>
<td>27–(54)–80</td>
<td>0–(0)–1</td>
</tr>
</tbody>
</table>

Note: The number in parenthesis indicates mean value.
In the case of bracteoles, the infraspecific taxa of claw to lamina in the wing and keel petals was 1:2–2.9.

Bracteoles of var. maximowiczii, thus, the length was generally approximately two times longer than the width. Bracteoles of var. maximowiczii var. maximowiczii, var. elongata also harbored longer calyx lobes (var. tomentella, 1.8–[3.1]–4.1 mm; var. elongata, 2.5–[3.0]–3.2 mm) than calyx tubes (var. tomentella, 1.4–[1.8]–2.2 mm; var. elongata, 1.2–[1.4]–1.6 mm). However, L. maximowiczii var. tricolor had shorter calyx lobes than calyx tubes (lobe, 0.7–[0.9]–1.1 mm; tube, 1.3–[1.5]–1.6 mm). The shape of the calyx lobes at the apex was acuminate for var. maximowiczii, var. tomentella, and var. elongata, whereas that of var. tricolor was acute, similar to that of L. buergeri. In all taxa, the ratio of claw to lamina in the wing and keel petals was 1:2–2.9. In the case of bracteoles, the infraspecific taxa of L. maximowiczii, except for var. tricolor, were ovate to elliptic; thus, the length was generally approximately two times longer than the width. Bracteoles of var. tricolor were also ovate, but the narrow form was not observed (width vs. length ≈ 1:1.7). Bracteoles of the Chinese L. buergeri generally were normal to broadly ovate, whereas those of Japanese individuals were almost round. Their ratio of width to length was similar to that of L. maximowiczii var. tricolor. Regarding hairs, the upper surface of the leaflets of L. maximowiczii was almost glabrous (1–[8]–59 per 4 mm²), whereas var. tomentella had more appressed hairs on the surface of leaflets (38–[51]–90 per 4 mm²). L. maximowiczii var. elongata also showed a similar density as the former two (27–[54]–80 per 4 mm²), whereas L. maximowiczii var. tricolor harbored almost glabrous and light green colored leaflets, similar to those of L. buergeri.

Regarding the hybrids, L. chiisanensis and L. maximowiczii showed similar flower structures. Flowers of L. patentibicolor and L. bicolor were also similar in shape (Fig. 3, Table 3). For standard petal, L. maximowiczii and L. chiisanensis had a distinct claw, whereas L. patentibicolor and L. bicolor had no obvious claw, which gradually decreased from the middle to bottom (Fig. 3). Wing petals of L. maximowiczii (6.4–[7.5]–9.2 mm) and L. chiisanensis (7.4–[8.3]–9.0 mm) were shorter than their keel petals (L. maximowiczii, 7.8–[8.8]–9.8 mm; L. chiisanensis, 8.8–[9.5]–10.7 mm). Although L. bicolor had longer keel petals (8.6–[10.1]–11.2 mm) than wing petals (8.6–[9.5]–11.0 mm), the difference in their length (mean 0.6 mm) was smaller than that of L. maximowiczii (mean 1.3 mm) and L. chiisanensis (mean 1.2 mm). In the case of L. patentibicolor, wing petals (7.1–[8.3]–8.9 mm) were longer than the keel petals (6.4–[7.6]–8.5 mm). For the ratio of claw to lamina in wing and keel petals, L. maximowiczii and L. chiisanensis had lamina

Table 3. Comparison of morphological traits of typical Lespedeza maximowiczii and hybrids with related species.

<table>
<thead>
<tr>
<th>Trait</th>
<th>L. maximowiczii</th>
<th>L. chiisanensis</th>
<th>L. patentibicolor</th>
<th>L. bicolor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of flower (mm)</td>
<td>8.2–(9.5)–10.9</td>
<td>10.2–(11.1)–12.0</td>
<td>8.1–(9.4)–11.5</td>
<td>10.5–(11.7)–12.7</td>
</tr>
<tr>
<td>Length of standard petal (mm)</td>
<td>7.0–(8.5)–10.0</td>
<td>9.6–(10.0)–10.3</td>
<td>8.9(9.2)–9.8</td>
<td>10.2–(11.3)–12.1</td>
</tr>
<tr>
<td>Length of standard petal claw (mm)</td>
<td>0.7–(1.1)–1.5</td>
<td>1.2–(1.5)–1.7</td>
<td>1.8–(2.0)–2.1</td>
<td>1.8–(2.0)–2.4</td>
</tr>
<tr>
<td>Width of standard petal (mm)</td>
<td>4.4–(5.6)–6.8</td>
<td>5.2–(5.8)–6.4</td>
<td>5.5–(5.6)–5.8</td>
<td>6.0–(7.7)–10.2</td>
</tr>
<tr>
<td>Claw of standard</td>
<td>Distinct</td>
<td>Distinct</td>
<td>No distinct</td>
<td>No distinct</td>
</tr>
<tr>
<td>Length of wing petal (mm)</td>
<td>6.4–(7.5)–9.2</td>
<td>7.4–(8.3)–9.0</td>
<td>7.1–(8.3)–8.9</td>
<td>8.6–(9.5)–11.0</td>
</tr>
<tr>
<td>Length of wing petal claw (mm)</td>
<td>1.7–(2.4)–2.9</td>
<td>2.4–(2.6)–3.0</td>
<td>2.5–(3.1)–3.8</td>
<td>3.2–(3.5)–3.7</td>
</tr>
<tr>
<td>Length of wing petal lamina (mm)</td>
<td>4.5–(5.4)–6.7</td>
<td>4.8–(5.8)–6.2</td>
<td>4.8–(5.3)–5.9</td>
<td>5.5–(6.1)–7.2</td>
</tr>
<tr>
<td>Length of keel petal (mm)</td>
<td>7.8–(8.8)–9.8</td>
<td>8.9–(10.3)–11.1</td>
<td>6.4–(7.6)–8.5</td>
<td>8.6–(10.1)–11.2</td>
</tr>
<tr>
<td>Length of keel petal claw (mm)</td>
<td>2.0–(2.6)–3.3</td>
<td>2.6–(2.9)–3.2</td>
<td>2.9–(3.3)–3.8</td>
<td>3.3–(3.7)–4.2</td>
</tr>
<tr>
<td>Length of keel petal lamina (mm)</td>
<td>5.6–(6.3)–7.0</td>
<td>6.5–(7.5)–8.1</td>
<td>3.7–(4.5)–5.7</td>
<td>6.5–(6.8)–7.2</td>
</tr>
<tr>
<td>Length of calyx tube (mm)</td>
<td>1.2–(1.5)–2.1</td>
<td>0.7–(2.1)–2.9</td>
<td>2.0–(2.3)–2.8</td>
<td>2.0–(2.2)–2.3</td>
</tr>
<tr>
<td>Length of calyx lobe (mm)</td>
<td>1.5–(2.3)–3.5</td>
<td>1.1–(2.1)–2.9</td>
<td>0.9–(1.2)–1.7</td>
<td>1.0–(1.4)–1.8</td>
</tr>
<tr>
<td>Shape of calyx lobe at apex</td>
<td>Mostly acuminate</td>
<td>Mostly acuminate</td>
<td>Mostly obtuse to round</td>
<td>Mostly obtuse to round</td>
</tr>
<tr>
<td>Shape of leaflets at apex</td>
<td>Mostly acute to acuminate</td>
<td>Acute to obtuse</td>
<td>Obtuse to emarginate</td>
<td>Obtuse to emarginate</td>
</tr>
</tbody>
</table>

Note: The number in parenthesis indicates mean value.
that was approximately two times longer than the claw, but the
lamina of *L. bicolor* and *L. patentibicolor* were not much longer
than the claw. Calyx lobes diverged below the middle of the
calyx in *L. maximowiczii* and *L. chiisanensis*, whereas the lobes
of *L. bicolor* and *L. patentibicolor* did not. The shapes of the
calyx lobes at their apex were also considerably different:
acuminate to acute in *L. maximowiczii* and *L. chiisanensis* and
obtuse to round in *L. bicolor* and *L. patentibicolor*.

**Genotypic analyses of Macrolespedeza taxa concerning *L. maximowiczii***

Regarding the Bayesian clustering results performed by
STRUCTURE, the optimum cluster number was determined
to be three because the highest $\Delta K$ was at this value (Fig.
4). At $K = 3$ (Fig. 5), most of *L. bicolor*, *L. patentibicolor*,
and *L. thunbergii* subsp. *thunbergii* were assigned to the
same lineage (orange). The infraspecific taxa of *L.*

---

maximowiczii (except for var. tricolor) and L. chiisanensis were assigned into the same lineage (light blue). Most of L. buergeri were grouped into a distinct lineage (dark purple), although some individuals appeared to be closer to L. maximowiczii. In the case of L. maximowiczii var. tricolor, all individuals appeared to have mixed genetic features from other infraspecific taxa (var. maximowiczii, var. tomentella, and var. elongata) and L. buergeri. Since \( K = 5 \) (Fig. 5), L. maximowiczii var. tricolor was assigned to an independent lineage (purple).

The NJ tree was almost consisted of the Bayesian clustering results (Fig. 6), despite this tree was weakly supported by low bootstrap value. Within clade A, L. thunbergii, L. bicolor, and L. patentibicolor were mainly clustered. The individuals of L. buergeri were included within clade B and C. In case of L. chiisanensis, this taxon was clustered with infraspecific taxa of L. maximowiczii (clade F and H).

**Discussion**

**Taxonomic reconsideration of infraspecific taxa of L. maximowiczii**

We examined the morphological traits of L. maximowiczii and its infraspecific taxa and analyzed their relationships using 11 microsatellite markers. Although morphological differences among L. maximowiczii var. maximowiczii, var. tomentella, and var. elongata were observed, the variation ranges mostly overlapped (Table 2). The width of leaflets showed a gradual change from narrow (represented as var. elongata) to wide (represented as var. maximowiczii). According to Nakai (1927), var. elongata is characterized by oblong or oblong-lanceolate leaflets that are acuminate at both ends. This leaflet form is also observed regardless of the presence of hair on the upper surface. L. maximowiczii var. tomentella bears hairs on the upper surface of leaflets, whereas the upper surface of the leaflet of var. maximowiczii becomes glabrous (Lee, 1965; Akiyama, 1988). Patent hair on inflorescences and branches
Fig. 6. Neighbor-joining phylogenetic tree of the infraspecific taxa of *Lespedeza maximowiczii* and related species based on microsatellite loci. The number above branch indicate bootstrap value (>50%). Voucher numbers are given in parentheses.
of var. tomentella has been suggested to be a specific trait (Lee and Lee, 1975) and this taxon has even been inferred to be a hybrid between L. maximowiczii and L. thunbergii (Lee and Lee, 1975). When we examined the specimens (ca. 1,100) that are deposited in KH to determine this hypothesis, patent hairs on inflorescences and branches were observed regardless of the pubescent on the upper surface of the leaflets. Appressed hairs on inflorescences were even observed in individuals bearing pubescent leaflets. Thus, we concluded that these two morphological traits were not correlated. In the Bayesian clustering results (Fig. 5), nearly all individuals of L. maximowiczii var. tomentella shared the same genetic lineage as L. maximowiczii var. maximowiczii; however, var. tomentella did not share a genetic lineage with L. thunbergii. Thus, L. maximowiczii var. tomentella could not be considered a hybrid, as suggested by Lee and Lee (1975). This result of the genetic analysis also applied to L. maximowiczii var. elongata (Fig. 5). As previously mentioned, L. maximowiczii var. elongata has been treated as a synonym for L. × friebena [= L. × tomentella in Lee (1980)] (Lee and Lee, 1975), including L. angustifolioides and L. patentielongata that were described as new hybrids in Lee (1965). We determined that L. angustifolioides and L. patentielongata were also not hybrids. However, L. maximowiczii var. tomentella was not morphologically different from var. maximowiczii, except for the hair on the upper surface of the leaflets (Fig. 2, Table 2). The pubescent form (var. tomentella) often grows with the glabrous form (var. maximowiczii) in the same place. Considering its resemblance with var. maximowiczii in both morphology and geographic distribution, var. tomentella should be ranked at the level of forma. However, the species epithet was changed because L. friebena preceded L. oldhamii var. tomentella Nakai (= L. maximowiczii var. tomentella) (Lee and Lee, 1975). Both taxa were observed to bear villous leaflets. Therefore, we named the forma L. maximowiczii f. friebena (Schindl.) D. P. Jin, J. W. Park & B. H. Choi.

However, L. maximowiczii var. tricolor is different from other infraspecific taxa in morphology. Its pale-yellow standard and keel petals appeared to be closer to L. buergeri. In the original description of this taxon (Nakai, 1923), the color of the keel petals was mentioned as rosy, but it is white or pale yellow in both field and herbarium specimens. In the order of the length of petals, L. maximowiczii var. tricolor (K > W ≒ S) resembles that of L. buergeri (K > W ≒ S) and some individuals even showed longer wing petals than standard petals (Table 2). Although length of wing and standard petals were similar in some individuals of L. maximowiczii, no individuals were observed to bear wing petals longer than the standard petals. Calyx lobes at the apex for var. tricolor (acute) were within the variation of L. buergeri (acute to obtuse). However, bracteoles of var. tricolor appeared close to those of var. maximowiczii because these taxa do not have the round shape that is regarded as the synapomorphy of L. buergeri (Akiyama, 1988; Jin et al., 2016b). Taking into account the intermediate features of var. tricolor and the migration history, we suggest that L. maximowiczii var. tricolor originated from an ancient hybridization between L. buergeri and L. maximowiczii (Jin et al., 2016b). Genotypes of var. tricolor were of mixed lineage of var. maximowiczii and L. buergeri (K = 3) (Fig. 5). In addition, most individuals of var. tricolor were determined to have a lineage that was distinguished from var. maximowiczii and L. buergeri from K = 5, which was supported by the low ΔK (Figs. 4, 5). Therefore, we treated L. maximowiczii var. tricolor as a distinct species, L. tricolor (Nakai) D. P. Jin, J. W. Park & B. H. Choi.

### Taxonomic reconsideration of hybrids between L. maximowiczii and its related taxa

Hybrids between L. maximowiczii and its related taxa were analyzed based on morphological and genetic traits. Although both L. patentiicolor (L. maximowiczii var. tomentella × L. bicolor) and L. chisanensis (L. maximowiczii × L. bicolor) are related to L. bicolor (Lee, 1965, 1980), their external appearance varies greatly (Figs. 1, 3). The flower of L. patentiicolor was within the morphological variation of L. bicolor. In detail, the length of claws and lamina of wing and keel petals of L. patentiicolor were approximately equal, and standard petals had no distinct claw, as does L. bicolor (Akiyama, 1988). In particular, Lee (1965, 1980) described the calyx lobes of L. patentiicolor as an intermediate form between its putative parents. However, our observations were opposed to what Lee (1965, 1980) suggested. The inflorescence of L. bicolor is generally glabrous (Lee, 1965, 1980; Akiyama, 1988), whereas that of L. patentiicolor is covered with hairs (Lee, 1965, 1980). The morphotype of L. patentiicolor could be considered L. bicolor var. sericea Nakai (Nakai, 1927), which has been treated as a synonym or forma of L. bicolor by other researchers (Hatusima, 1967; Akiyama, 1988). L. patentiicolor could be also be regarded as L. melanantha Nakai, characterized by semicircular calyx lobes at the apex and wing petals longer than keel petals (Nakai, 1927; Akiyama, 1988). Although the shape of the calyx is different in both
taxa, the order of the petals in length is the same for both (Lee, 1978). In the case of *L. melanantha*, the width of the calyx lobes is longer than their length (Akiyama, 1988); however, morphological variation must be examined. In Bayesian clustering analysis (Fig. 5), most of *L. patentibicolor* were assigned to the same lineage as *L. bicolor*, but they did not share a genetic lineage with *L. maximowiczii* var. *tomentella*. On the NJ tree (Fig. 6), *L. patentibicolor* was clustered into a clade with *L. bicolor* and *L. thunbergii*, supporting the result of the Bayesian clustering analysis. Although the discussion regarding the relationship among infraspecific taxa of *L. bicolor* and taxonomic delimitation of *L. melanantha* is limited in this study, based on our data, we believe that *L. patentibicolor* is circumscribed into *L. bicolor*.

*L. chisianensis* has acute to obtuse leaflets (or emarginate) at the apex and acuminate calyx lobes (Lee, 1965). Its inflorescence is thin, similar to that of *L. bicolor* (Lee, 1965). However, *L. chisianensis* bears appressed hair, similar to that of *L. maximowiczii* (Lee, 1965). However, these characters were not well separated from *L. maximowiczii*. Although the inflorescence of some specimens was thin in the upper part, the lower part was not different from that of the common *L. maximowiczii*. The corolla of *L. chisianensis* resembled that of *L. maximowiczii* rather than *L. bicolor* (Fig. 3, Table 3). Standard petal of *L. chisianensis* showed definite claw and auricular shapes that were similar to those of *L. maximowiczii*. Acuminate calyx lobes appeared to be typical of *L. maximowiczii*. Additionally, the wing and keel petals were in the form of *L. maximowiczii*, i.e., the lamina was two times longer than the claw. In Bayesian clustering analysis (Fig. 5), the individuals, identified as *L. chisianensis*, were grouped into *L. maximowiczii*. Hence, both morphological and genetic traits suggested that *L. chisianensis* was within the variation of *L. maximowiczii*. When considering these facts, it is likely that this hybrid should be treated as a synonym of *L. maximowiczii*.

A key to *Lespedeza maximowiczii* and its related taxa

1. Standard and keel petal purple, standard longer than wing petal in length; calyx acuminate and elongated at apex; leaflets glabrous or pubescent on upper surface, usually green to dark green. "*Lespedeza maximowiczii*

2. Bracteoles elliptic to ovate; leaflet apex usually acute to acuminate. "*L. tricolor*

A key to the forma of *Lespedeza maximowiczii*

1. Leaflet upper surface pubescent during fruiting season, sparsely hairy during flowering season. "*f. maximowiczii*

2. Bracteoles elliptic to ovate; leaflet apex usually obtuse to acute. "*L. buergeri*

**Taxonomic Treatment**


A Korean name: Jo-rok-ssa-ri (조록싸리).

Shrub, erect, up to 2.5 m tall, much branched at upper part. Bark brown to grayish-brown, eftled when old. Branches terete, glabrous, reddish brown to brown, stretched slightly in zigzag pattern; young branches with appressed or patent hairs. Stipules linear triangular to linear, brown, 2–3 mm long. Leaves (based on upper branches) trifoliate, petiolate, stipulate, usually larger at lower part of plant; petioles 9–25 mm long, with sparse to dense appressed to patent hairs; rachises 5.5–15.5 mm long, with sparse to dense appressed or patent hairs; terminal leaflets green to dark green, petiolulate; petiolules ca. 2 mm long, swollen, appressed to patent hair; lamina 3–6 cm long, 1–4 cm wide, elliptic to broad ovate, entire, acute to acuminate (to obtuse) at apex, rounded or obtuse (to acuminate) at base, upper surface with sparsely appressed hairs during flowering season, becoming glabrous during fruiting, lower surface with more densely appressed hairs; lateral leaflets slightly smaller than terminal. Inflorescence axillary pseudo-raceme, usually one (rarely two or three) per leaf, similar length with subtending leaf, 3–7 cm long, 12–18 (to 32) flowered, with appressed to
and SNUA are identified as this forma in KH, on a specimen collected from Nangok (North Korea, Gangwon-W).


*L. angustifoloides* T. B. Lee, Bull. Seoul Nat. Univ. For. no. 2: 3, 1965. *pro parte, quoad specim*. Tanyang (= Danyang-gun), Jeechon (=Jeechon-gun), Yeonkok (= Yeongok, Jirisan Mt., Gurye-gun).—TYPE: KOREA. Chungcheongbuk-do: Danyang-gun, 21 Jun 1964, T. Lee s.n. (lectotype, designed here, SNUA, see Fig. 1C).

*L. patentielongata* T. B. Lee, Bull. Seoul Nat. Univ. For. no. 2: 26, 1965. *excl. specim*. Temple Soodeuk (= Temple Sudeoksa, Yesan-gun).—TYPE: KOREA. Jeollanam-do: Gurye-gun, Pia valley, around Yeongok, 23 Jul 1963, T. Lee s.n. (holotype, SNUA!, see Fig. 1D).

**Korean name:** Teol-jo-rok-ssa-ri (털조록싸리).

This form bears adpressed hairs on the surface of leaflets until the fruiting period.

**Taxonomic note:** During the flowering period, this taxon is hardly distinguished from *f. maximowiczii* because the latter could also bear adpressed hair on the upper surface of leaflets during that time. However, the upper surface of leaflets in *f. friebeana* is generally more pubescent than *f. maximowiczii* (>51 hairs per 2 mm²) (Table 2). Except for hairs on upper surface of leaflets, the morphological characters of *f. friebeana* are included in the variation of *f. maximowiczii*. Lee mentioned that patent hair on the inflorescence is an important key to identify this taxon when considering Schindler’s description (Lee 1965; Lee and Lee 1975). The patent hair on the inflorescence is usually observed in *f. friebeana*, but this character is sometimes observed in *f. maximowiczii*. Therefore, caution is required, when this form is identified based on this character.

This form has been known as *tomentella* since Nakai named it as *L. oldhamii* Miq. var. *tomentella* Nakai (Nakai, 1919). Although he mentioned *L. tomentella* Nakai based on the hairs of leaflets in his previous study (1914), he stopped publishing *L. tomentella*. This was because he knew *f. friebeana* before the publication of *L. tomentella* (Nakai, 1914). *Lespedeza friebeana* was described based on Wilford’s collection (Schindler, 1911), which bears sparse hairs on the upper surface of leaflets. The hairs of the form are not different from those of *L. maximowiczii* var. *tomentella* (including *L. oldhamii* var. *tomentella*) (Nakai, 1919, 1927), and this was written in the original description. In addition, *f. friebeana* (Schindler, 1911) predate *L. oldhamii* var. *tomentella* (Nakai, 1919). The history of the species epithet was according to Lee and Lee (1975). Hence, we chose *f. friebeana* as the epithet of the form that bears sparse hairs on the upper surface of leaflets.

Shrub, erect, 1–2 m tall, much branched at the upper part. Bark light brown to gray, vertically clefted when old. Branch terete, glabrous, light brown to brown, sometimes stretched in a zigzag pattern; young branches with appressed hairs. Stipules linear triangular to linear, brown, 2–3 mm long. Leaves trifoliolate, petiolate, stipulate, usually bigger at lower part of plant; petioles 9–30 mm long, with appressed hairs; rachises 9–20 mm long, similar to petioles; terminal leaflets green, petiolululate; petiolules ca. 2 mm long, swollen, with densely appressed hairs; lamina 3–7 cm long, 2–4 cm wide, elliptic to ovate, entire, acute to acuminate (to obtuse) at apex, rounded or obtuse (to acute) at base, upper surface glabrous, lower surface pubescent; lateral leaflets similar to terminal ones but relatively smaller in size. Inflorescence axillary pseudo-raceme, usually one (rarely two or three) per leaf, 2.6–8 cm long, similar or smaller in size. Inflorescence axillary pseudo-raceme, usually one (rarely two or three) per leaf, 2.6–8 cm long, similar or smaller in size. Inflorescence axillary pseudo-raceme, usually one (rarely two or three) per leaf, 2.6–8 cm long, similar or smaller in size. Inflorescence axillary pseudo-raceme, usually one (rarely two or three) per leaf, 2.6–8 cm long, similar or smaller in size.
2.8 mm wide, pale yellow to white (similar or paler than standard petal); lamina 5.4–6.4 mm long, slightly falcate, obtuse at apex, sometimes purple dotted at apex; claw 2.2–2.9 mm long. Stamens 10, similar to keel petal in length, diadelphous (9 + 1), white; filaments equal in length; anthers elliptic, retuse at both sides, ca. 0.4 mm long, ca. 0.2 mm wide, yellow. Pistil as long as stamen, with appressed hairs from base to middle, glabrous at apex; style elongated above anther; ovary narrow elliptic during flowering season, hairy. Legume slightly distorted elliptic, compressed, subsessile, ca. 13 mm long, ca. 5 mm wide, pilose, elongated spine-tip. Seed reniform, ca. 5 mm long, ca. 3 mm wide, glabrous.

**Flowering:** July–August.

**Fruiting:** September–October.

**Distribution:** Restricted to Korea (south-western regions of Jeollanam-do). Endemic.

**Taxonomic note:** This species resembles *L. buergeri* in color of flowers, order of petal length, shape of calyx, and glabrous upper surface of leaflets. However, the bracteole of each taxon is different. *L. tricolor* bears an ovate to elliptic form, whereas *L. buergeri* bears a round to elliptic form. In addition, bracteoles of *L. buergeri* could almost cover the calyx tube. Apex of leaflets of *L. tricolor* are generally acute to acuminate, but that of *L. buergeri* are usually obtuse to acute. Nakai (1923, 1927) and other researchers (Lee, 1965; Akiyama, 1988) mentioned that *L. tricolor* bears rosy keel petals, but our examination found that the color of keel petals was similar or paler than that of standard petal. On the other hand, *L. maximowiczii* has a purple standard petal, acute to acuminate leaflet apex, and elliptic bracteoles. Although *L. maximowiczii* is distinct from *L. tricolor* in the color of standard petal, the former is similar to the latter in the shape of leaflets and bracteoles. Regarding genetic structure, *L. tricolor* harbors a mixed genetic lineage between *L. buergeri* and *L. maximowiczii* ($K = 3$) (Fig. 5), even *L. tricolor* shows an independent lineage from the latter two ($K = 5$) (Fig. 5). In a previous study (Jin et al.,
2016b), *L. tricolor* was originated from *L. buergeri* that had migrated from Japan, which experienced gene flow with *L. maximowiczii* in Korea during the Quaternary. Therefore, we considered *L. tricolor* as a distinct species from *L. maximowiczii* and *L. buergeri*.


**ORCID**: Dong-Pil JIN https://orcid.org/0000-0002-3617-952X; Jong-Won PARK https://orcid.org/0000-0002-5877-4696; Byyoung-Hee CHOI https://orcid.org/0000-0002-9234-9052

**Acknowledgments**

The authors are grateful to the persons concerned at the KH, SNUA, PE and TI herbaria for permitting the examination of specimens. We also thank our colleague J.S. Park, Plant Systematics Laboratory of Inha University, for commenting on this manuscript and editing the figures. This work was supported by the National Research Foundation of Korea (NRF) (No. NRF — 2018R1D1A1B07043030).

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**Literature Cited**


조록싸리 종내분류군과 잡종의 분류학적 연구

진동필·박종원·최병희*
인하대학교 생명과학과


주요어: 잡종, 종내분류군, 조록싸리, 형태 변이, microsatellite, 분류학적 처리