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ORIGINAL RESEARCH

Major Lineages of the Genus *Lilium* (Liliaceae) Based on nrDNA ITS Sequences, with Special Emphasis on the Korean Species

Chang Shook Lee · Seung-Chul Kim · Sung Hee Yeau · Nam Sook Lee

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Abstract We present most comprehensive phylogenetic analysis of 196 accessions of *Lilium* representing 83 species and 14 varieties of *Lilium* and three outgroup genera (*Cardiocrinum*, *Notholirion*, and *Fritillaria*) to investigate infrageneric relationships within *Lilium* as well as to determine the origin and evolution of Korean species of *Lilium*. We used the internal transcribed spacer sequences of nuclear ribosomal DNA and phylogenetic analysis using maximum parsimony and Bayesian inference identified several major lineages within *Lilium*. Only one section, *Martagon*, turned out to be monophyletic in the study. Three sections, *Archelirion*, *Liriotypus*, and *Pseudolirium*, are not monophyletic because two, one, and two species in each section were placed in other lineage, respectively. Two major lineages of section *Leucolirion* were confirmed in this study, and as several previous studies suggested, section *Sinomartagon* is highly polyphyletic. The origin of *Lilium hansonii*, a Korean endemic to Ullung Island, is perplexing given the fact that it has ribotype of *Martagon*, while its cpDNA haplotype is similar to *Sinomartagon*. The origin of another endemic, *Lilium amabile*, is equally elusive and additional phylogenetic and phylogeographic studies will shed light on their evolutions in Korea. We determined that *Lilium callosum* var. *flavum* originated from *L. callosum* in Southern Korea.

Keywords ITS · Korean *Lilium* · *Lilium* · Liliaceae · Major lineages

C. S. Lee
Institute of Eco Science, Ewha Womans University,
Seoul 120-750, South Korea

S.-C. Kim (✉)
Department of Biological Sciences, Sungkyunkwan University,
300 Cheoncheon-dong, Jangan-gu,
Suwon, Gyeonggi-do 440-746, South Korea
e-mail: sonchus96@skku.edu

S. H. Yeau
Department of Science Education, Ewha Womans University,
Seoul 120-750, South Korea

N. S. Lee (✉)
Department of Life Science, Ewha Womans University,
Seoul 120-750, South Korea
e-mail: namsook@ewha.ac.kr

The genus *Lilium* L. consists of approximately 100 species that are widely distributed throughout the cold and temperate regions of the northern hemisphere (Krause 1930; McRae 1998). Southwestern and Himalayan Asia-China are considered the center of diversity (ca. 70 species) for the genus (Lighty 1968; Baranova 1969). Lilies are perennial plants with subterranean bulbs and are mostly spring-flowering plants growing in steppes and mountain meadows. The bulbs generally have numerous imbricate fleshy scales, and stem is often multifoliate with alternate or verticiallate leaves at several levels. The flowers are few or numerous and are borne in racemose or sometimes umbellate inflorescences, or they may be terminal and solitary. They are mostly large and magnificent, funnel-shaped, often with more or less recurved tepals, which vary in color from white to yellow, orange, bright red, or dark purple, the inner side being mostly spotted and sometimes supplied with hairs. Because of large, showy, and often strongly fragrant flowers, the genus *Lilium* has long been attracted horticulturalists and incalculable number of breeding studies resulting hybrids have been conducted. Phylogenetic relationships among species of *Lilium* have been proposed based on the results of numerous

interspecific hybridization studies (e.g., Lighty 1960, 1968; Asano 1986; Noda 1987)

Infrageneric treatment of the genus *Lilium*, however, has long been problematic and controversial. For example, Baker (1871) divided the genus into four sections (*Eulirion* Rchb., *Archelirion* Baker, *Isolirion* Baker, and *Martagon* Rchb.) primarily based on flower shape. Later, Wilson (1925) adopted the Baker's treatment, with an inclusion of additional character (i.e., the position of anthers), recognized four sections. Based on the 13 morphological characteristics and two germination types, Comber (1949) proposed the most authoritative and comprehensive classification of the genus, recognizing seven sections: *Martagon* Rchb., *Pseudolirium* Endl, *Liriotypus* Ascj. and Graebn., *Archelirion* Baker, *Sinomartagon* H. F. Comber, *Leucolirion*, and *Daurolirion* H. F. Comber.

The infrageneric treatment of Comber (1949) of *Lilium* has been evaluated by several molecular phylogenetic studies. Nishikawa et al. (1999) conducted the first comprehensive molecular phylogenetic analysis of genus *Lilium* using the internal transcribed spacer (ITS) of nuclear ribosomal DNA. They included 55 species of *Lilium* and determined their phylogenetic relationships among them. The results indicated that three of seven sections, *Archelirion*, *Pseudolirium*, and *Martagon*, are monophyletic. Two lineages of *Leucolirion* were identified; one lineage (6a) is sister to *Lilium henryi* (sect. *Sinomartagon*, 5a), while the other lineage (6b) is sister to *Lilium brownii* (sect. *Archelirion*) and is nested within *Sinomartagon*. The largest section in the genus, *Sinomartagon* (ca. 30 species), is highly polyphyletic, and section *Daurolirion* is paraphyletic. Section *Liriotypus* turned out to be not monophyletic because of the position of *Lilium bulbiferum* L.; this species is closely related to section *Daurolirion*. Overall, most internal branches and supports for some clades were not significant in the maximum-likelihood tree. Nishikawa et al. (2001) further evaluated phylogenetic relationships of section *Sinomartagon* based on the ITS of nrDNA from a total of 64 *Lilium* species. They found five lineages of *Sinomartagon*, confirming their previous results, in which *Sinomartagon* is highly polyphyletic. In this study, Nishikawa et al. (2001) found that earlier monophyletic section *Archelirion* is no longer monophyletic (*L. brownii*, additionally sampled species in this study, is sister to *Leucolirion*). Only two of seven sections, *Pseudolirium* (26% bootstrap support) and *Martagon* (97% bootstrap support), turned out to be monophyletic. Again, most internal branches were very weakly supported in this study.

In addition to the large-scale phylogenetic studies of the genus *Lilium*, several regional phylogenetic studies were also conducted. For example, Dubouzet and Shinoda (1999) used the same ITS sequences to determine phylogenetic relationships among 16 species of Japanese *Lilium*. In this study, the phylogenetic position of *Lilium dauricum* Ker-Gawler was determined; this species is closely related to those of section *Sinomartagon*. The phylogenetic relationships among the Japanese species were also fine-tuned. The origins and genome size evolution of European lilies were also investigated (Ikinci et al. 2006; Muratovic et al. 2010). All but one species (i.e., *Lilium martagon* in section *Martagon*) of European lilies belong to section *Liriotypus* (Comber 1949), and their phylogenetic relationships were determined based on ITS sequences. Ikinci et al. (2006) have shown that section *Liriotypus* is not monophyletic; again, *L. bulbiferum* is more closely related to other sections than to its own section. Muratovic et al. (2010) found three major lineages within European lilies: *L. martagon* group (PP=100%), *L. bulbiferum* (PP=100%), and the third group with the remaining species of the Comber's *Liriotypus* section (PP=100%). Two significant conclusions were drawn with regard to identifying major lineages within *Lilium*: (1) *L. bulbiferum* is not part of section *Liriotypus* and (2) *L. martagon* L. and *Lilium cattaniae* Vis. from section *Martagon* are not related to endemic lilies from section *Liriotypus*, in contrary to the statement of Reichenback (1830), Baker (1871), or Wilson (1925). These regional phylogenetic studies provided some insights into understanding the major lineages in the genus *Lilium*. However, it became apparent that a broad scale phylogenetic analysis including all the species studied earlier in regional scale as well as several unpublished species (i.e., ITS sequences of *Lilium* deposited in GenBank and other databases) is required to identify major lineages within *Lilium* and also to evaluate infrageneric classification.

Very little is known about phylogenetic relationships among Korean species of *Lilium*. Approximately 11 species and two varieties of *Lilium* are distributed in Korea (Flora of Korea Editorial Committee (The Genera of Vascular Plants of Korea 2007)). Two species (*L. hansonii* and *L. amabile*) and one variety (*L. callosum* var. *flavum*) exclusively occur in Korea, and the remaining species are distributed widely in eastern Asia (Japan, China, and Russia). Species in Korea belong to either section *Martagon* or *Sinomartagon*. Only handful of studies was conducted to investigate the relationships among species in Korea. For example, Kim and Lee (1990a, b, c) conducted morphological and anatomical studies of Korean *Lilium*. They demonstrated that two sections are morphologically and anatomically quite divergent and also that section *Martagon* is more primitive than *Sinomartagon*. These studies, however, provided very little phylogenetic relationships among the species within each section. Later, Lee et al. (1993) conducted randomly amplified polymorphic DNA (RAPD) analysis, representing the first and only molecular study but did not provide any additional insights into the phylogenetic relationships among Korean *Lilium* species. Therefore, it is necessary to conduct comprehensive phylogenetic study of Korean species to

determine their phylogenetic relationships as well as to understand their relationships relative to other species in *Lilium*.

The objective of this study is twofold. First, we hoped to find and recognize major lineages within the genus *Lilium*. Although several previous studies addressed this issue, the current study represents the first most comprehensive sampling of *Lilium* (i.e., 83 of approximately 100 species and 14 varieties), and we utilized both maximum parsimony and Bayesian inference. Recognizing major lineages within the genus also allowed us to evaluate infrageneric classifications and phylogenetic relationships among sections within the genus. Second, we wanted to determine phylogenetic relationships among species of *Lilium* in Korea. In particular, we assessed the phylogenetic position and origin of Korean endemic species.

Materials and Methods

Sampling, DNA Extraction, PCR, and Sequencing Reaction Approximately 11 species and two variety of *Lilium* are distributed widely in Korea (The Genera of Vascular Plants of Korea 2007). Of these, two species and one variety (*L. callosum* var. *flavum*, *L. hansonii*, and *L. amabile*) are endemic to Korea, while all other taxa occur primarily in eastern Asia (Japan, China, and Russia). All the species in Korea belong to sections *Martagon* (*Lilium distichum*, *L. hansonii*, and *Lilium tsingtauense*) and *Sinomartagon* (*L. amabile*, *L. callosum*, *L. callosum* var. *flavum*, *Lilium cernuum*, *Lilium concolor*, *Lilium lancifolium*, and *Lilium leichtlini*). We sampled a total of 16 populations representing nine species and one variety and sequenced 38 accessions (Table 1).

Total genomic DNA from fresh/dry leaf tissue was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The nuclear rDNA ITS region was amplified using primers ITS5 and ITS4 of (White et al. 1990) and PCR conditions are the same as described in Kim et al. (1996) and Suh et al. (2002). Amplification products were purified using Qiaquick purification kit (Qiagen, Valencia, CA, USA). Sequencing reactions were carried out for the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, CA, USA). Sequencing primers used were identical to amplification primers (ITS4 and ITS5) and two additional internal primers (ITS2 and ITS3) were used.

Sequence Editing and Alignment For the sequences we generated in this study, sequence fragments were assembled and edited using Sequencher version 4.2.2 (Gen Codes, Ann Arbor, MI, USA). We also obtained all the other sequences of *Lilium* deposited in GenBank and other databases. We used Clustal X (Thompson et al. 1997) to align the entire sequences based on the default parameters.

Phylogenetic Analysis The ITS dataset, a total of 196 accessions representing 83 species (including one undescribed species, *L.* sp 2NC1) and 14 varieties, was analyzed using an equally weighted, unordered maximum parsimony (MP) approach (Fitch 1971) implemented in PAUP version 4.0 (Swofford 2002). We used three genera, *Cardiocrinum giganteum* (Wall.) Makino, *Notholirion bulbuliferum* (Lingl.) Stearn, and *Fritillaria camtschatcensis* (L.) Ker-Gawl., as outgroups based on the previous studies (Nishikawa et al. 1999; Hayashi and Kawano 2000). The MP analysis included a default heuristic search for the most parsimonious trees: starting trees were obtained via stepwise addition. Sequences were added via simple addition with one tree held at each step. Branch swapping was performed via tree-bisection-reconnection (TBR), and steepest descent and MulTrees options were in effect. Branches were collapsed if maximum branch length was zero, and topological constraints were not enforced. Bootstrap support (BS) was calculated by bootstrap analysis from 1,000 bootstrap replicates (Felsenstein 1985) with the same heuristic options.

Bayesian inference of the ITS dataset was implemented using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Likelihood parameters for Bayesian analysis were calculated using MrModeltest 2.2 (Nylander 2004). "GTR+Γ" model was chosen under Akaike Information Criterion. The Bayesian Markov chain Monte Carlo algorithm was run for 1,000,000 generations with four simultaneous chains (three "cold" and one "heated"), starting from random trees and sampling every 100 generations. We discarded as burn-in the first 20% of the total number of generations. We generated a 50% majority-rule consensus tree from the remaining trees, in which the percentage of nodes recovered presented their posterior probability (PP). The following scale for BS percentages was used: 50–74%, low; 75–84%, moderate; 85–100%, strong. For Bayesian clade support estimates, good support was considered for $PP \geq 0.90$ and low to no support for $PP \leq 0.89$.

For phylogenetic analysis of Korean *Lilium* species, we analyzed the smaller data matrix (71 taxa including two outgroups) including all the Korean *Lilium* species and other related species from sections *Martagon*, *Sinomartagon*, *Daurolirion*, *Leucolirion*, *Liriotypus*, *Pseudolirium*, and *Archelirion*. We conducted reduced dataset analysis because the larger dataset analysis was highly unresolved among species in Korea, which was possibly caused by collapsed branches among topologically different parsimonious trees in construction of the strict consensus tree. Two species (i.e., *L. amoenum* E. H. Wilson ex Stoker and *L.*

Table 1 Species of *Lilium* distributed in Korea used in this study

Species	DNA accession number	Locality	GenBank accession
Section *Martagon* Rchb.			
L. distichum Nakai ex Kamib.	16	Gaam-ri, Nam-myeon, Damyang, Jeollanam Province	HQ223039
	17	Gaam-ri, Nam-myeon, Damyang, Jeollanam Province	HQ223040
	18	Gaam-ri, Nam-myeon, Damyang, Jeollanam Province	HQ223041
	19	Gaam-ri, Nam-myeon, Damyang, Jeollanam Province	HQ223042
	20	Gaam-ri, Nam-myeon, Damyang, Jeollanam Province	HQ223043
L. hansonii Leichtlin ex Baker	57	Ulleung Island, Gyeongsangbuk Province	HQ223044
	58	Ulleung Island, Gyeongsangbuk Province	HQ223045
	59	Ulleung Island, Gyeongsangbuk Province	HQ223046
	60	Ulleung Island, Gyeongsangbuk Province	HQ223047
L. tsingtauense Gilg	1	Mt. Daeam, Gangwon Province	HQ223048
	7	Mt. Daedeog, Gangwon Province	HQ223049
	9	Mt. Daedeog, Gangwon Province	HQ223051
	55	Mt. Deogyu, Gyeongsangnam Province	HQ223052
	56	Mt. Deogyu, Gyeongsangnam Province	HQ223053
Section *Sinomartagon* H. F. Comber			
L. amabile Palib.	2	Mt. Daeam, Gangwon Province	HQ223054
L. callosum Siebold and Zucc.	34	Hoesu-dong, Seogwipo-si, Jeju Province	HQ223055
	35	Hoesu-dong, Seogwipo-si, Jeju Province	HQ223056
	39	Masan-ri, Dado-myeon, Naju, Jeollanam Province	HQ223057
	40	Masan-ri, Dado-myeon, Naju, Jeollanam Province	HQ223058
	41	Masan-ri, Dado-myeon, Naju, Jeollanam Province	HQ223059
	147	Mt. Halla, 900 m, Jeju Province	HQ223060
	148	Mt. Halla, 900 m, Jeju Province	HQ223061
L. callosum Siebold and Zucc. var. *flavum* Y. Lee	152	Cheongwon, Chungcheongbuk Province	HQ223062
L. cernuum Kom.	27	Mt. Daedeog, Gangwon Province	HQ223063
	28	Mt. Daedeog, Gangwon Province	HQ223064
	29	Mt. Daedeog, Gangwon Province	HQ223065
	141	Mt. Seokbyung, Gangwon Province	HQ223066
	142	Mt. Seokbyung, Gangwon Province	HQ223067
L. concolor Salisb.	3	Geumdaebong, Gangwon Province	HQ223068
	4	Geumdaebong, Gangwon Province	HQ223069
	5	Geumdaebong, Gangwon Province	HQ223070
	6	Geumdaebong, Gangwon Province	HQ223071
L. lancifolium Thunb.	30	Masan-ri, Dado-myeon, Naju, Jeollanam Province	HQ223074
	31	Masan-ri, Dado-myeon, Naju, Jeollanam Province	HQ223073
	32	Masan-ri, Dado-myeon, Naju, Jeollanam Province	HQ223072
	50	Sambong beach, Ahnmyeondo, Chungcheongnam Province	HQ223075
	54	Mt. Bongrae, Jeollanam Province	HQ223076
L. leichtlinii Hook. f.	33	Unknown	HQ223077

wardii Stapf ex W. W. Sm.) from one lineage of section *Sinomartagon* based on our analysis of larger dataset were chosen as outgroups. Optimal models of molecular evolution were chosen, using the likelihood ratio test (Goldman 1993; Whelan and Goldman 1999) implemented in ModelTest 3.7 (Posada and Crandall 1998). Model parameters were then imported into PAUP*, and a heuristic search (asis sequence addition, TBR branch swapping, and MULPARS option on) was executed. Maximum likelihood (ML) bootstrap analyses with 100 replicates were conducted, using the same parameter values obtained from the ModelTest and heuristic options.

Results

A total of 642 aligned characters were used for phylogenetic analysis. We found 171 constant characters (26.6%), 178 variable parsimony uninformative characters (27.7%), and 293 parsimony informative characters (45.6%) including outgroups. The heuristic search resulted in more than 140,000 trees (we stopped the search when the maxtree reached 140,000 due to insufficient memory), with a tree length of 1654, consistency index of 0.4601 (0.3733 excluding uninformative characters), and a retention index of 0.7557. The strict consensus tree is shown in Fig. 1. Because the tree topologies resulting from Bayesian analysis recovered essentially the same as those resolved using MP, only the MP tree (strict consensus) with nodal support indicated by both BS and PP values are presented here.

Several major clades found in the previous studies (e.g., Nishikawa et al. 1999, 2001) were also found in this analysis. Lack of resolutions and poor supports among major lineages are apparent. Nevertheless, identified major clades are as follows (we used the original groups identified by Nishikawa et al. (1999)): (1) Group I (BS 75%, PP 0.99), section *Leucolirion* and *L. henryi* Baker; (2) Group II (BS 91%, PP 1.00), section *Archelirion*; (3) Group III (BS <50%, PP 0.95), section *Pseudolirium* plus *L. humboldtii* var. *bloomerianum* Purdy; (4) Group IV (BS 88%, PP 1.00), section *Liriotypus*; (5) Group V (BS 99%, PP 0.85), section *Martagon*; (6) Group VI (BS <50%, PP <0.50), sections *Sinomartagon, Leucolirion*, and *Daurolirion* plus *L. bulbiferum*, *L. brownii* var. *viridulum* Baker, and *L. brownii* var. *colchesteri* Wils.

In terms of phylogenetic relationships among the major lineages, section *Leucolirion* (Group I, 6a in Nishikawa et al. 1999) shares its most recent common ancestor with section *Archelirion* (Group II; BS<50%, PP 0.99). These two lineages and section *Liriotypus* (Group IV) without any further resolutions formed the basal lineages within *Lilium* (Fig. 1a). Next lineage is section *Pseudolirium* (Group III, BS<50%, PP 0.95) and this lineage shares its most recent common ancestor with some members of polyphyletic section *Sinomartagon* and recently described section *Lophophorum* (Bur. and Franch.) F. T. Wang and T. Tang (with very week or no support; Fig. 1b). The last lineage is strongly supported (BS 88%, PP 1.00) including Groups V and VI (Fig. 1c). Within this lineage, well-supported monophyletic section *Martagon* (Group V) is sister to the clade (Group VI) containing some members of section *Leucolirion* (6b in Nishikawa et al. 1999), section *Daurolirion* and majority of section *Sinomartagon*. This sister relationship between Groups V and VI is, however, very weakly supported (BS<50%, PP 0.59).

All species of *Lilium* in Korea belong to either section *Martagon* or *Sinomartagon*. Three species, *L. hansonii*, *L. tsingtauense*, and *L. distichum*, belong to monophyletic section *Martagon*, and the strict consensus tree shows that *L. distichum* shares its most recent common ancestor with *L. tsingtauense* (Fig. 1c). Five individuals (from one population) of *L. distichum* have all different ribotypes, while only two types were found in five individuals (from three populations) of *L. tsingtauense*. Since the ITS consensus tree was not well resolved within section *Martagon*, the origin of *L. hansonii*, endemic to Ullung Island in Korea, was not determined precisely in this analysis. However, the ITS tree, at least, suggests that *L. hansonii* is closely related to *L. martagon*, *Lilium medeoloides*, and *L. cattaniae*. In case of the species in section *Sinomartagon*, the phylogenetic relationships were highly unresolved, like within section *Martagon*. The strict consensus tree, however, suggests that *L. callosum* is closely related to *L. concolor* and two other species (i.e., *Lilium tigrinum* and *Lilium leichtlinii*). Four ribotypes were found in *L. callosum*, but those ribotypes were not necessarily correlated with their geographical origins. Only one ribotype was found in *L. concolor*, while two ribotypes were found from two populations of *L. cernuum*.

Model test selected "TrN+I+G" as the best-fit model based on the likelihood ratio test, and the ML analysis found two trees, which are identical except in the branch lengths. One of two ML trees is shown in Fig. 2. Like in the strict consensus tree of genus *Lilium* (Fig. 1), the ML tree for Korean *Lilium* species was not well resolved, and most internal branches were supported with relatively low bootstrap values, with an exception of one clade for section *Martagon* (BS 99%). Two major lineages were recognized: one with the species in section *Martagon* (BS 99%) and the other with the species in section *Sinomartagon* (BS 62%). The ML tree shows that *L. hansonii*, endemic to Ullung Island in Korea, is closely related to *L. martagon* and several other species (see more details in "Discussion"). Two species in section *Martagon*, *L. tsingtauense* and *L. disticum*, which occur in lower elevations in mountains of Korea, are closely related (however, one GenBank accession EU303295 with relatively long branch length is very oddly closely related to *L. hansonii*). For *L. amabile*, another endemic in southern part of Korean peninsula, its origin seems uncertain based on ITS sequences, but the results suggest that the species is closely related to *Lilium davidii* Duch., *Lilium pumilum* de Candolle, and *L. cernuum*. The third Korean endemic, *L. callosum* var. *flavum*, has identical ribotype as *L. callosum*, especially from the populations in Jeju Island and Southern Korean Province.

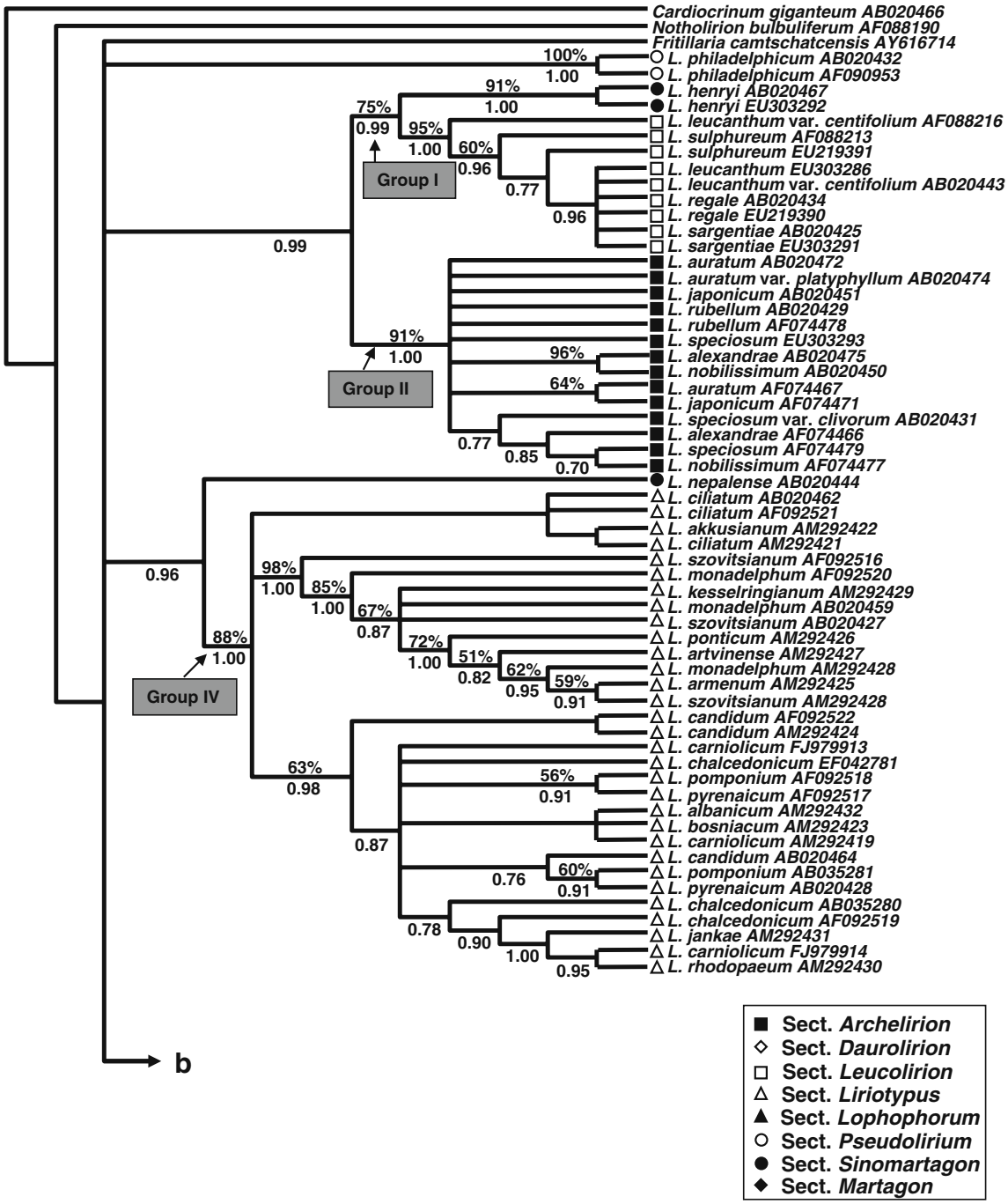

Fig. 1 Strict consensus tree of genus *Lilium* based on nrDNA ITS sequences. Bootstrap values and posterior probabilities are shown *above and below branches*, respectively. Different shapes were used for sectional treatments of Comber (1949) (taxon without shape represents "not classified") and the groups are based on (Nishikawa et al. 1999, 2001). Species in *gray* represent the taxa sampled in this study (i.e., additional samples of *Lilium* species from Korea)

Discussion

Major Lineages within Lilium One of the two objectives of this study was to identify and recognize major lineages within the large genus *Lilium*, and we addressed this object based on nrDNA ITS sequences. Given the availability of large number of ITS sequences in various databases for 83 of nearly 100 species in the genus, our comprehensive analysis in this study allowed us to address this object effectively. We confirmed several important findings that earlier studies revealed (e.g., Dubouzet and Shinoda 1999; Nishikawa et al. 1999, 2001; Hayashi and Kawano 2000; Rønsted et al. 2005; Ikinci et al. 2006; Muratovic et al. 2010). We discuss several important findings in this study and confirmation of the

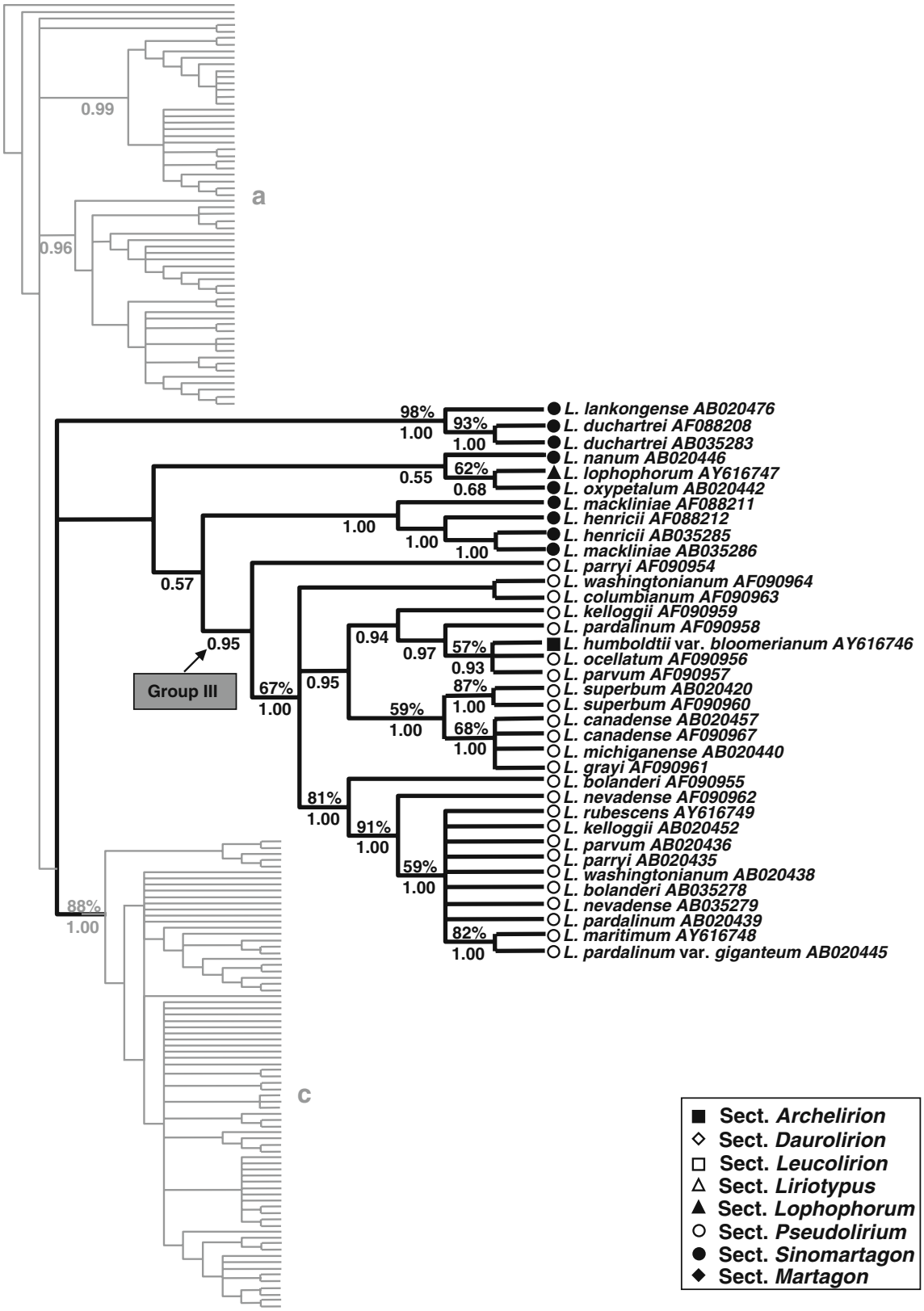

Fig. 1 (continued)

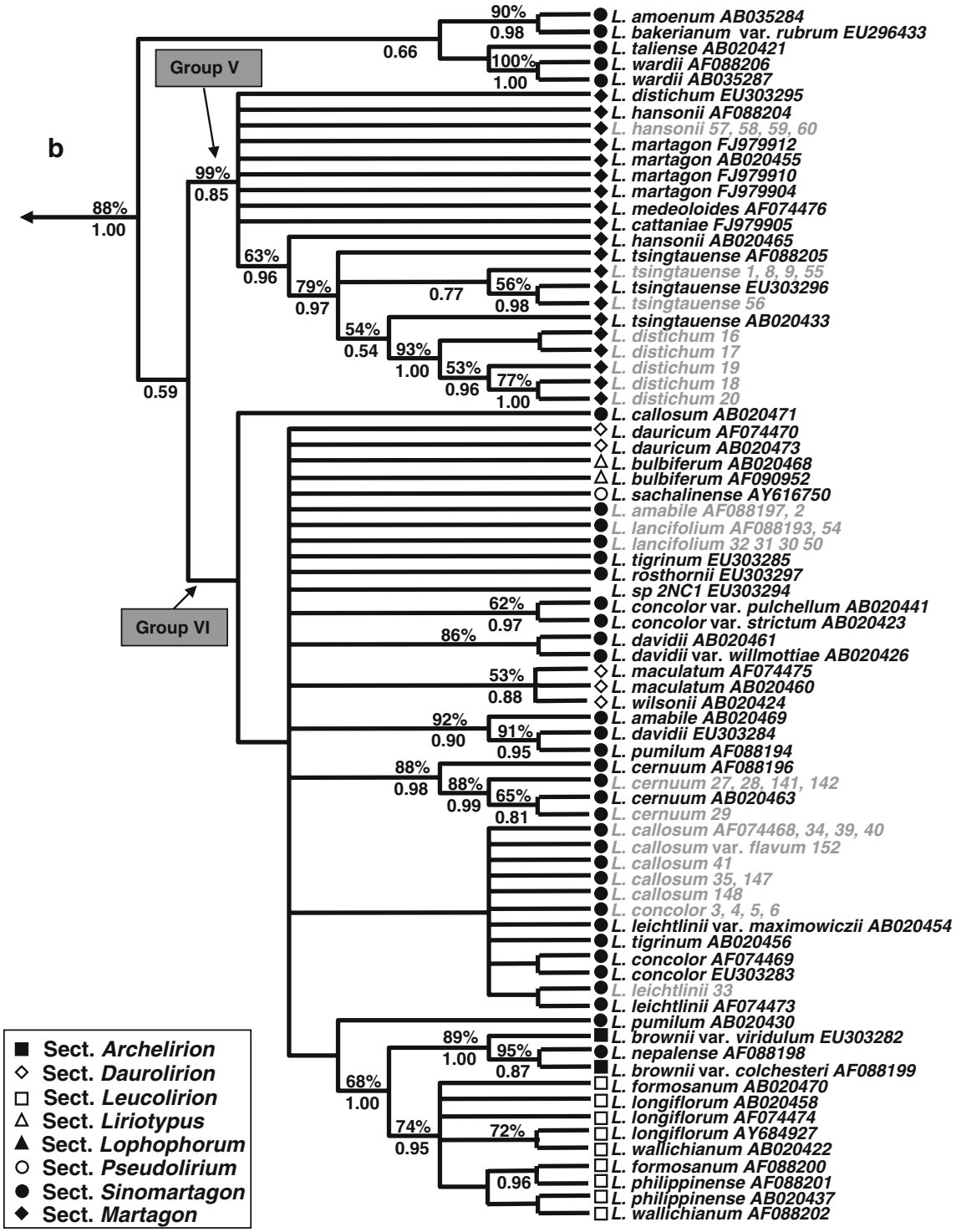

Fig. 1 (continued)

previous studies below. The groups defined originally by Nishikawa et al. (1999) were adopted in this discussion and also in figures:

Group I—it becomes clear that *L. henryi* in section *Sinomartagon* is closely related to one of two lineages of section *Leucolirion* (Fig. 1a). The sister relationship between them is rather strongly supported (BS 75% and PP 0.99). *L. henryi*, an endemic to three provinces in Central China (Guizhou, Hubei, and Jiangxi), is morphologically similar to *L. speciosum* Thunberg (*Archelirion*, subsection 4a) and *L. rothornii* Diels

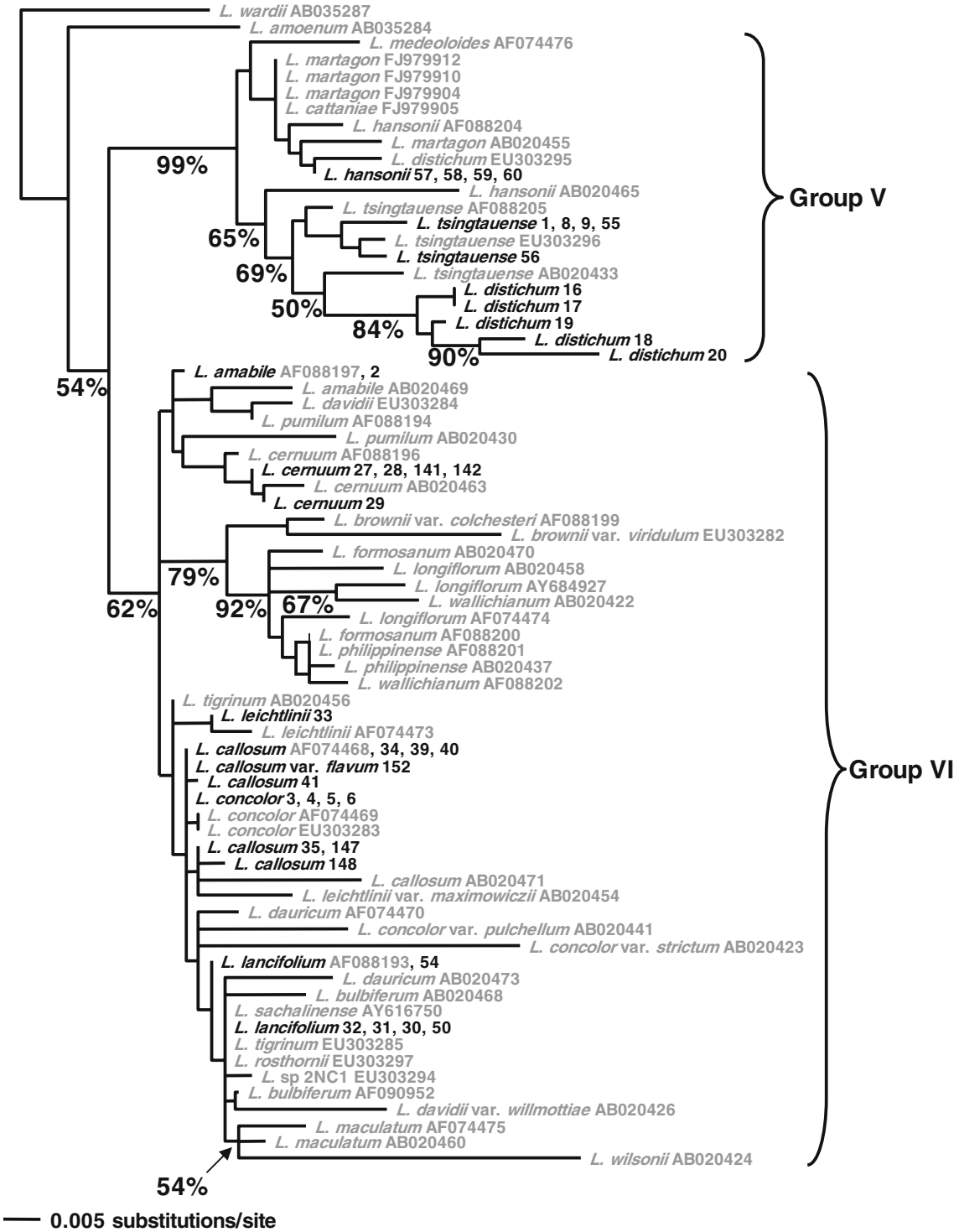

Fig. 2 One of two ML trees (-lnL=3200.6294) for the phylogenetic analysis of Korean *Lilium* species. Bootstrap values above 50% are shown *below branches*. The species sampled in the current study are shown in *black*. The groups are based on (Nishikawa et al. 1999, 2001)

(*Sinomartagon*, subsection 5a). *L. henryi*, however, has unusual features in seed characteristics from sect. *Sinomartagon* and also in bulb size and color from subsection 5a, suggesting a distinct lineage from section *Sinomartagon*. Seed fertility also suggested that *L. henryi* is more closely related to section *Leucolirion* (6a) than to *Sinoartagon*; thus, *L. henryi* and section *Leucolirion* (6a) were classified horticulturally into the same Division VI (Leslie 1982). We also found that *L. sulphureum* Baker ex Hook. F.

belongs to this lineage, and the cytological study (Smyth et al. 1989) supported close relationships among the species within this clade. Group I is sister to Group II (PP 0.99).

Group II—this group is comprised of the Japanese species only classified as section *Archelirion* (Fig. 1a). This section is usually characterized based on hypogeal delayed germination (excluding *L. brownii* and *L. speciosum*), scattered leaves, erect stem, stem roots, and white bulbs. We confirmed the earlier findings by Dubouzet and Shinoda (1999), and the current results further confirmed that *L. brownii* from section *Archelirion* is closely related to the species of section *Leucolirion* (Fig. 1c, Group VI). Excluding two taxa, *L. brownii* and *L. humboltii* var. *bloomerianum*, section *Archelirion* is monophyletic.

Group III—this group (PP 0.95) includes the members of the New World taxa, section *Pseudolirium* (Fig. 1b; Lighty 1968). Morphologically, this section can be characterized based on hypogeal delayed germination, whorled leaves, joined bulb scales (with several exceptions), heavy seeds (with several exceptions), erect stem, and rhizomatous to stoloniferous bulbs. The phylogenetic position of *L. philadelphicum* L. is elusive since the Group III includes all but *L. philadelphicum* from the North America. The ITS tree (Fig. 1a) strongly suggests that *L. philadelphicum* is not part of *Pseudolirium* and its position is uncertain; this taxon represents one of several lineages (i.e., Groups I, II, and IV) radiated early in the evolution of *Lilium*. This unusual and uncertain position of *L. philadelphicum* is corroborated by chloroplast *mat*K and *rbc*L gene sequence data (Hayashi and Kawano 2000). In addition, *L. philadelphicum* is the only species that has no cross ability with any other North American species (Lighty 1968). Therefore, crossing experiments and molecular phylogenetic studies strongly suggest that *L. philadelphicum* is a distinct lineage from *Pseudolirion* and should be placed in a new and separate section, as argued by Hayashi and Kawano (2000). In addition to *L. philadelphicum*, the sectional placement of *L. humboltii* var. *bloomerianum* (California endemic) in *Archelirion* is not supported in this study; this taxon is closely related to *L. ocellatum* [syn. *L. humboldtii* ssp. *ocellatum* (Kellogg) Thorne] and *L. parvum* Kellogg (PP 0.93; BS 57%) in section *Pseudolirion*. Lastly, unusual placement of *L. sachalinense*, rare, endemic to the island of Sakhalin (E Russia) in the North American section *Pseudolirion* is also not supported in this study; this taxon is part of highly unresolved lineages in Group VI (Fig. 1c; also see discussion below).

Group IV—this group represents one of highly supported clades in *Lilium* (BS 88% and PP 1.00) and includes most European lilies in section *Liriotypus* (Fig. 1a). Traditionally, section *Liriotypus* can be characterized based on epigeal delayed germination (excluding *L. bulbiferum*, *Lilium polyphyllum*, and *Lilium monadelphum*), scattered leaves, numerous bulb scales, heavy seeds, and erect stems. The three major lineages of the European lilies are also confirmed by this study (Nishikawa et al. 1999, 2001; Ikinci et al. 2006; Muratovic et al. 2010). *L. bulbiferum* as a distinct lineage from section *Liriotypus* is unquestionable, but its phylogenetic position within *Lilium* is uncertain even in this study (Fig. 1c, Group VI). *L. bulbiferum* was placed in various sections of *Lilium* (e.g., Baker 1871; Wilson 1925; Baranova 1988), and several molecular phylogenetics studies suggested its relationship relative to species in other section (e.g., close relationship between *L. bulbiferum* and section *Dauriolirion* (Nishikawa et al. 1999, 2001)). Our current study suggests that it is closely related to some species of sections *Sinomartagon* and *Daurolirion* and *L. sachaliense* (Fig. 2). The chromosome pattern of this species is completely different from other species in section *Liriotypus* (Muratovic et al. 2010). Although the distribution of *L. bulbiferum* in Europe is far from that of *Daurolirion* and *Sinomartagon* in Eastern Asia, it is well hybridized with *Daurolirion* and *Sinomartagon*, suggesting close relationships between them (McRae 1998). The precise phylogenetic position of *L. bulbiferum* and its origin in Europe, however, require further investigation.

Group V—this groups represent section *Martagon* and unlikely what Lighty (1968) suggested, it is one of well-supported monophyletic groups (Fig. 1c; BS 99% and PP 0.85). The monophyly of this section is further supported by cross ability among the species (Shimizu 1971) and their narrow and limited distribution to Eastern Asia (except for *L. martagon*). Several features traditionally defined section *Martagon* include hypogeal delayed germination, whorled leaves, jointed bulb scales (excluding *L. hansonii*), heavy seeds, and flowers with small and waxy tepals. *L. martagon*, as well as *L. cattaniae*, a taxon with uncertain taxonomic status, clearly belong to the highly supported clade of section *Martagon*, and they are not related to other European lilies from section *Liriotypus* (as suggested by Reichenback 1830; Endlicher 1836; Baker 1871; Wilson 1925). The cytogenetic data by Muratovic et al. (2010) further supported distinct nature of these two taxa from *Liriotypus*.

Group VI—although this group includes few unusually placed taxa (e.g., *L. bulbiferum* from *Liriotypus*, *L.*

sachalinense from *Pseudolirium*, and *L brownii* from *Archelirion*), it includes all members of section *Daurolirion* and majority members of section *Sinomartagon*. This group also includes the second lineage of *Leucolirion* (6b of Nishikawa et al. 1999). The monophyly of section *Daurolirion* require further test, but highly polyphyletic nature of *Sinomartagon* is highlighted again in this study. The polyphyly of *Sinomartagon* suggest that some of the characters of Comber (1949) (i.e., immediate epigeal germination, scattered leaves, entire bulb scales, light seeds, turk's cap flowers, erect white bulbs, more or less stoloniform stems, stem roots, and small stigma) for delimiting this section are highly homoplasious.

Phylogenetic Relationships among Korean Lilium sp. The origin of Korean endemic lilies and phylogenetic relationships among Korean species were, for the first time, assessed in this study. First of all, the origin of Ullung Island endemic, *L. hansonii*, is puzzling. No explicit hypothesis on the origin of *L. hansonii* has been postulated, although several studies suggested its close relationship to *L. distichum* and *L. tsingtauense* (e.g., Woodcock and Stearn 1950; Lee 1989; Lee et al. 1993; Sultana et al. 2010). We found only one ribotype based on four individuals in our collections (Table 1 and Fig. 2). This ribotype is quite different from two other accessions (AF088204 and AB020465; Nishikawa et al. 1999), although one accession (AF088204) is closely related to the ribotype of our collections. We were not able to confirm independently about the identity of these GenBank accessions due to lack of voucher information. It is yet to be determined whether these represent intraspecific variation of ribotypes, paralogous repeats, or different taxa. Nevertheless, the ITS tree suggests that *L. hansonii* shares its most recent common ancestor with *L. martagon*, *L. cattaniae*, and *L. distichum*, judging from our materials of *L. hansonii*. It seems less likely that *L. cattaniae* involved in the origin of *L. hansonii* in Ullung Island since *L. cattaniae* is narrow endemic to Croatia and Bosnia. It is uncertain whether *L. distichum* also involved in the origin of *L. hansonii* because this accession from China (EU303295) is very different from five ribotypes collected in Korea (these five ribotypes belong to different clade; Fig. 2). This leaves only one species as candidate, i.e., *L. martagon*, and since this species does not occur in Korea, involvement of this species on the origin of *L. hansonii* is questionable. Next plausible candidate species are *L. tsingtauense* and *L. distichum* from the same section *Martagon* (Fig. 2. Group V). *L. tsingtauense* occurs widely in all provinces in Korea, especially in thickets and forests in lower elevations of mountains. *L. distichum* also occurs in all provinces in Korea, especially mountain forests in high elevations. Morphologically, *L. distichum* and *L. tsingtauense* are more closely related to each other than either one is to *L. hansonii*. This close relationship between *L. distichum* and *L. tsingtauense* is corroborated by RAPD data (Lee et al. 1993) and physical mapping of rRNA loci (Sultana et al. 2010).

The situation becomes even more complicated when we consider the chloroplast DNA. Hayashi and Kawano (2000), based on *mat*K gene sequences, found that several species of section *Sinomartagon*, i.e., *L. callosum*, *L. lancifolium*, *L. leichtlinii* var. *maximowczii*, *L. maculatum* Thunb., and *L. maculatum* ssp. *dauricum* (Baker) Hara, have very similar cpDNA haplotype as *L. hansonii*; only up to two mutations were found between *L. hansonii* and these four species. On the contrary, 4–7 mutations were found between *L. hansonii* and section *Martagon*. More specifically, there is only one substitution between *L. hansonii* and *L. maculatum* ssp. *dauricum*. Of the five species in section *Sinomartagon*, all but *L. maculatum* species occur in Korea. Thus, the molecular data suggest that *L. hansonii* has the chloroplast haplotype of section *Sinomartagon*, whereas it has the nuclear ribotype of section *Martagon*. Species within section *Martagon* are well hybridized (Shimizu 1971), but we do not know whether species of sections *Martagon* and *Sinomartagon* are reproductively isolated. We could not provide any perceivable explanation about possible involvement of these two sections in the origin of *L. hansonii* and additional phylogenetic studies would clarify this perplex origin of this species in Ullung Island.

The origin of other endemic, *L. amabile*, also seems to be unclear. This species occurs in central and southern part of Korean peninsula, especially in the mountain forests in lower elevations. Morphologically, *L. amabile* is closely related to *L. lancifolium*, *L. leichtlinii* var. *maximowczii*, *L. cernuum*, and *L. pumilum* in Korea (Lee 1989; Kim and Lee 1990a), but it can be distinguished from them based on bulb and leaf shapes. The ITS tree (Fig. 2) suggests that it is closely related to *L. pumilum*, *L. cernuum*, and *L. davidii*; two species, *L. lancifolium* and *L. leichtlinii* var. *maximowczii*, are distantly related to *L. amabile*. Both *L. cernuum* and *L. pumilum* occur in Korea (*L. pumilum* is restricted to North Korea); thus, it is plausible that these two species were involved in the origin of *L. amabile*. *L. davidii* occurs widely in moist places in forests, forests margins, and grassy slopes in central to southwestern provinces of China; thus, we cannot rule out the possibility about involvement of this species in the origin of *L. amabile*. All three possible progenitor species (i.e., *L. pumilum*, *L. cernuum*, and *L. davidii*) occur in higher elevations, while *L. amabile* occurs in lower elevations in mountain forests. Two morphologically similar species, *L. leichtlinii* var. *maximowczii* and *L. lacifolium*, occur widely

in lower elevations of mountain forests in Korea. Due to short internal branch lengths and low BS supports among the lineages in this major clade (i.e., sections *Sinomartagon* and *Daurolirion* and one lineage of *Leucolirion*), the potential role of these two species needs to be investigated. No molecular data other than ITS are available to address the origin of *L. amabile* and additional molecular phylogenetic studies will shed light on its origin.

Lastly, with regard to the origin of *L. callosum* var. *flavum*, which is endemic to Jeollanam province, it has the same ribotype as *L. callosum* from Jeju Island and southern part of the Korean Peninsula. Thus, it is likely that it was originated from *L. callosum* in southern part of Korean Peninsula. Interestingly, four ribotypes (out of seven individuals from three populations in Jeju Island and Jeollanam province) were found in *L. callosum*, and this ribotype is identical to Korean individuals of *L. concolor* (only one ribotype is present). These two species occur in different elevations (*L. callosum* and *L. concolor* in low and high elevations, respectively) but are widely sympatric. It is yet to be determined whether sharing the same ribotype is due to sharing the same common ancestry or recent gene flow (i.e., hybridization or introgression).

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