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On the origin of European lilies: phylogenetic analysis of *Lilium* section *Liriotypus (Liliaceae)* using sequences of the nuclear ribosomal transcribed spacers

Abstract

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The sequences of the nuclear ribosomal internal transcribed spacer (ITS) region were analysed for 28 representatives of *Lilium,* one *Nomocharis* species and three outgroup taxa of *Lilieae (Notholirion, Fritillaria* and *Cardiocrinum).* 17 of the 20 members of *Lilium* sect. *Liriotypus* were included. A maximum parsimony analysis was carried out for the phylogenetic reconstruction. The results are not completely congruent with sectional delimitations of *L.* sect. *Liriotypus* based on morphological characters. They confirm the previous suggestion that *L.* sect. *Liriotypus* is monophyletic only if *L. bulbiferum* is excluded and placed in *L.* sect. *Sinomartagon*. The monophyly of the remaining *L.* sect. *Liriotypus* receives good support from bootstrap analysis. It can be divided into two groups, one comprising NE Turkish-Caucasian species and another the European species, *L. candidum* and the two Turkish endemics *L. ciliatum* and *L. akkusianum*. The results also show that *L. ponticum* cannot be included within the so-called *L. carniolicum* group of lilies.

Key words: ITS, phylogeny, systematics, DNA sequencing, Turkey, Caucasia.

Introduction

The genus *Lilium* L. includes approximately 100 species distributed mainly in temperate regions throughout the northern hemisphere. Infrageneric classification of *Lilium* is subject of considerable discussion and the number of sections used differs depending on the author. Based on flower shape, Baker (1871) divided *Lilium* into four sections: *L.* sect. *Eulirion* Rchb. (funnel-flowered lilies), *L.* sect. *Archelirion* Baker (open-flowered lilies), *L.* sect. *Isolirion* Baker (erect-flowered lilies) and *L.* sect. *Martagon* Rchb. (Turk's cap lilies). Again using flower shape, but also using the position of the anthers, Wilson (1925) divided the genus into four sections similar to those of Baker's classification. The difference between the classification of the two authors is that Wilson (1925) includes in *L.* sect. *Archelirion* only *L. auratum,* the type species of this section, while he placed the other members of Bakers's (1871) section into *L.* sect. *Martagon*. Comber (1949) reclassified the genus *Lilium* into seven sections using 15 diagnostic characters, not only of the flower and the growth habit, but also of the germination and bulb structure.

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Table 1. Sectional classification according to different authors of the European, NE Turkish and Caucasian *Lilium* species included in this study.

Taxon	Baker (1871)	Wilson (1925)	Comber (1949)	Baranova (1988)
L. akkusianum R. Gämperle	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. albanicum Griseb.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. armenum Grossh.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. artvinense Miscz.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. bosniacum G. Beck	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. bulbiferum L.	*Isolirion*	*Pseudolirium*	*Liriotypus*	*Pseudolirium*
L. candidum L.	*Eulirion*	*Leucolirion*	*Liriotypus*	*Lilium*
L. carniolicum Bernh.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. ciliatum P. H. Davis	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. jankae A. Kern.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. kesselringianum Miscz.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. martagon L.	*Martagon*	*Martagon*	*Martagon*	*Martagon*
L. monadelphum M. Bieb.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. pomponium L.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. ponticum K. Koch	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. pyrenaicum Gouan	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. rhodopaeum Delip.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*
L. szovitsianum Fisch. & Avé-Lall.	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*

According to Comber (1949), *Lilium* sect. *Liriotypus* has a total number of 20 species and includes all European, Turkish and Caucasian species (Fig. 1) except for *L. martagon,* which has the widest distribution range of all *Lilium* species and belongs to *L.* sect. *Martagon* Rchb. (Table 1). Since no lilies are distributed between Asia Minor / Caucasus and E Afghanistan (Stern 1938), the section thus contains all but one *Lilium* species occurring west of this gap. With the exception of *L. candidum* with widely trumpet-shaped flowers and *L. bulbiferum* with erect bowl-shaped flowers, all members of *L.* sect. *Liriotypus* have scattered leaves and Turk's cap flowers (McRae 1998).

Baranova (1988) further subdivided the genus into eleven sections and classified the European lilies into four different sections (Table 1). She placed *L. bulbiferum* in *L.* sect. *Pseudolirium* E. H. Wilson with other Asian and American erect-flowering lilies and *L. candidum* in the unispecific section *Lilium*. *L. martagon* is placed in *L.* sect. *Martagon* together with other lilies having a verticillate leaf arrangement. The remaining European lilies with Turk's cap flowers and scattered leaf arrangements were placed in *L.* sect. *Eurolirium* Baranova.

All members of the genus *Lilium* have the same basic chromosome number (x = 12), similar chromosome morphology and a very large genome size (Siljak-Yakovlev & al. 2003). Smyth & al. (1989) analysed 20 *Lilium* species from six sections and showed that C-banding patterns can provide only little information for deducing the relationships between species.

Recently, several molecular markers have proved useful in developing an improved classification. Yamagishi (1995) employed the RAPD technique to differentiate *Lilium* species and hybrids. Hayashi & Kawano (2000) used the coding regions of *rbc*L and *mat*K genes of chloroplast DNA for their phylogenetic analysis of the family *Liliaceae* sensu stricto and also for the infrageneric relationships within *Lilium,* although due to low substitution rates, with the *rbc*L gene the resolution was very low. The *mat*K marker proved to be more variable than the *rbc*L gene. The study of these latter authors, however, included only four representatives of the *L.* sect. *Liriotypus* and these four species were not resolved as a clade from other closely related sections.

Dubouzet & Shinoda (1999) employed the sequences of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA to resolve the phylogenetic relationships among Japa-

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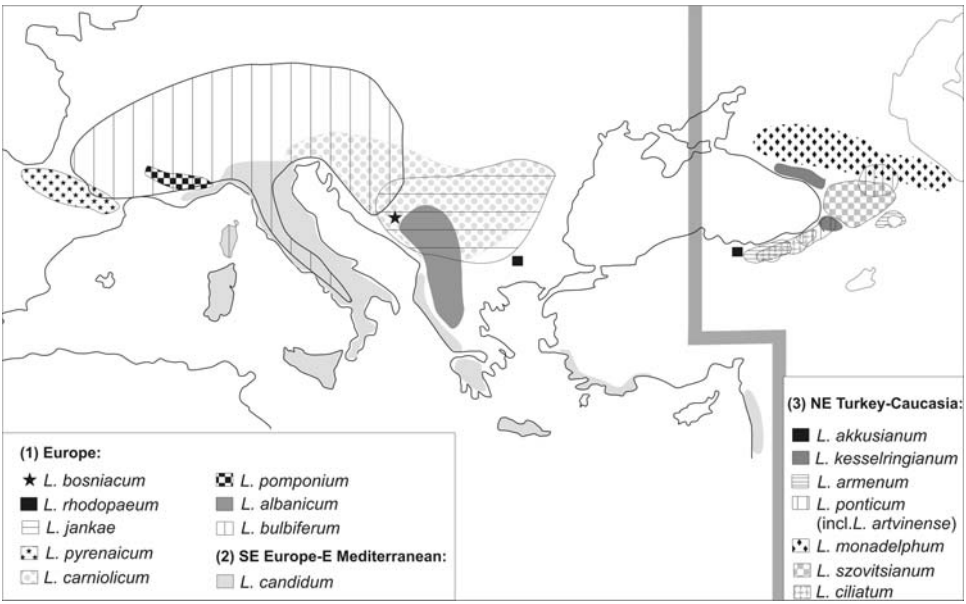


Fig. 1. Distribution of the European, Turkish and Caucasian species of *Lilium* sect. *Liriotypus* included in this study according to Davis & Henderson (1970), Stoker (1938, 1939), Synge (1980), Matthews (1980) and personal observations.

nese *Lilium* species and suggested further use of this region in a broader sampling of taxa. Their results were congruent with Comber's (1949) morphological classification. However, no representatives of *L.* sect. *Liriotypus* were included because they do not occur in that area. At the same time Nishikawa & al. (1999) showed that the nrDNA ITS region is a useful marker to unravel the phylogenetic relationships and section delimitations of *Lilium*. Their study of 55 species of *Lilium* included representatives of all sections according to the classification by Comber (1949) but only six species of the European *L.* sect. *Liriotypus*. The latter section did not form a monophyletic group, because *L. bulbiferum* was shown to be more closely related to *L.* sect. *Daurolirion* than to the other members of *L.* sect. *Liriotypus*.

The present paper analyses the phylogenetic relationships of *Lilium* sect. *Liriotypus* based on a phylogenetic analysis of sequence variation of the nuclear ribosomal ITS region with a comprehensive sampling representing 17 of the section's 20 members. It aims at a better understanding of the biogeography and evolution of *L.* sect. *Liriotypus* and of the origin of the European lilies. Following the taxonomic view of Popova (1966) and Muratović (2005) rather than of Mathews (1980), we recognise *L. bosniacum, L. jankae* and *L. albanicum* as distinct species and not as varieties of a polymorphic *L. carniolicum*.

The present authors performed also a preliminary study with the sequences of the *trn*L (UAA) intron and the intergenic spacer region between the *trn*L (UAA) 3' exon and the *trn*F (GAA) gene of the chloroplast DNA on nine species of *Lilium* sect. *Liriotypus*. Amplifications were performed using the universal primers of trnl-c, trnl-d, trn-e and trnl-f of Taberlet (1991). However, it was seen that there is no variable nucleotide position for a phylogenetic reconstruction. Therefore these chloroplast sequence data were not included in our analysis.

Material and methods

Plant material. – Sequences of the ITS region of 29 *Lilium* species (including *Nomocharis*) representing all sections of the genus sensu Comber (1949), as well as *Notholirion thomsonianum*

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Table 2. List of the taxa and the sources of the plant material or of the sequences, respectively, included in the analysis.

Taxon	Source and EMBL / GenBank accession number
Lilium L.	
sect. *Archelirion* Baker	
L. auratum Lindl.	Nishikawa & al. (1999) — AB020472
L. japonicum Thunb.	Nishikawa & al. (1999) — AB020451
sect. *Daurolirion* H. F. Comber	
L. dauricum Ker Gawl.	Nishikawa & al. (1999) — AB020473
sect. *Leucolirion* Wilson	
L. longiflorum Thunb.	Yang, C. M. & Chen, R. S. (unpubl.) — AY684927
L. philippinense Baker	Nishikawa & al. (1999) — AB020437
sect. *Liriotypus* Asch. & Graebn.	
L. akkusianum R. Gämperle	Turkey, Ordu, Akkuş, 23.6.2002, *İkinci 1928* (AIBU) — AM292422
L. albanicum Griseb.	Albanien-Montenegro, 29.6.1914, *Dörfler 432* (WU) — AM292432
L. armenum Grossh.	Turkey, Trabzon, Tonya, 25.6.2002, *İkinci 1933* (AIBU) — AM292425
L. artvinense Miscz.	Turkey, Artvin, 2.7.2002, *İkinci 1960* (AIBU) — AM292427
L. bosniacum G. Beck	Croatia, Velebit, Karlobag, *B. Zollitsch* (M 0056366) — AM292423
L. bulbiferum	Nishikawa & al. (1999) — AB020468
L. candidum L.	Turkey, Muğla, Köyceğiz, 2.6.2002, *İkinci 1912* (AIBU) — AM292424
L. carniolicum Bernh.	Austria, Königsberg, 1988 (M 0056392) — AM292419
L. ciliatum P. H. Davis	Turkey, Giresun, Tamdere, 25.6.2002, *İkinci 1932* (AIBU) — AM292421
L. jankae A. Kern.	Romania, Transsilvania, *J. Cstaó* (B) — AM292431
L. kesselringianum Miscz.	Turkey, Artvin, Ardanuç, 3.7.2002, *İkinci 1966* (AIBU) — AM292429
L. monadelphum M. Bieb.	Russia, Stawropol, Kislowodsk, 29.5.1970, *coll. ignot.* (JE) — AM292418
L. pomponium L.	Nishikawa & al. (1999) — AB035281
L. ponticum K. Koch	Turkey, Trabzon, Çaykara, 28.6.2002, *İkinci 1944* (AIBU) — AM292426
L. pyrenaicum Gouan	Nishikawa & al. (1999) — AB020428
L. rhodopaeum Delip.	Greece, Rhodopi Mt, 23.7.1981, *Strid & al. 19503* (B) — AM292430
L. szovitsianum Fisch. & Avé-Lall.	Turkey, Ardahan, Çıldır, 5.7.2002, *İkinci 1973* (AIBU) — AM292428
sect. *Martagon* Rchb.	
L. martagon L.	Nishikawa & al. (1999) — AB020455
sect. *Pseudolirium* Endl.	
L. canadense L.	Nishikawa & al. (1999) — AB020457
L. philadelphicum L.	Nishikawa & al. (1999) — AB020432
sect. *Sinomartagon* H. F. Comber	
L. nepalense D. Don	Nishikawa & al. (1999) — AB020444
sect. *Sinomartagon* H. F. Comber	
L. pumilum DC	Nishikawa & al. (1999) — AB020430
L. davidii Duch.	Nishikawa & al. (1999) — AB020461
Nomocharis Franch.	
Nomocharis saluenensis Balf.	Nishikawa & al. (1999) — AB020449
Outgroups	
Cardiocrinum giganteum (Wall.) Makino	Nishikawa & al. (1999) – AB020466
Fritillaria latifolia Willd.	Turkey, Artvin, Borkça, 6.7.2002, *İkinci 1977* (AIBU) — AM292420
Notholirion thomsonianum (Royle) Stapf	Rønsted & al. (2005) – AY616752

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(Royle) Stapf, *Fritillaria latifolia* Willd. and *Cardiocrinum giganteum* (Wall.) Makino were used (Table 2). Leaf material was collected in the field and dried in silica gel; in addition leaf probes were prepared from herbarium specimens.

DNA isolation, amplification and sequencing. – Using the protocol provided by the manufacturer, total DNA was extracted from 20-25 mg of silica-gel dried leaves or leaves from herbarium material using Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The ITS region was amplified in two pieces due to usage of herbarium material, since the degraded DNA was easier to amplify in short pieces. The ITS1 region was amplified by using the primers ITS5 (White & al. 1990) and ITS1P2, which was designed by Ochsmann (2000) for *Centaurea* L. ITS2 was amplified using the primers ITS3 and ITS4 designed by White & al. (1990). Amplification conditions were 94 °C (2 min) initial denaturation, 40 cycles of 94 °C (20 s) denaturation, 55 °C (45 s) annealing, 72 °C (1 min) elongation and 72 °C (10 min) final elongation. PCR products were purified by Montage PCR Centrifugal Filter Device (Millipore Company). Cycle sequencing of purified PCR products was performed by using the CEQ Dye Terminator Cycle Sequencing Start Kit (Beckman Coulter) and sequences were analysed in a CEQ 8000 automated sequencer (Beckman Coulter). All new nrDNA ITS sequences were submitted to the EMBL sequence data bank (accession numbers given in Table 2).

Sequence analysis. – Limits of the ITS-1, the 5.8S rRNA gene and ITS-2 were determined by comparison with published sequences. DNA sequences were aligned using ClustalX (Thompson & al. 1997) and the alignment was corrected manually, following the guidelines by Kelchner (2000).

Phylogenetic reconstruction. – Maximum parsimony analyses of the data set were performed using the heuristic search algorithm of PAUP* version 4.0b10 (Swofford 2002) with ACCTRAN; MULPARS and tree-bisection-reconnection (TBR) branch swapping in action. 1000 addition sequence replicates were performed to locate potential islands of the most parsimonious trees. Character states were specified unordered and unweighted and gaps in aligned sequences were treated as missing data. The transitions/transversions ratio was set equal. A bootstrap analysis was performed for the measurement of clade support with the following settings: 100 bootstrap replicates with 10 random addition sequence replicates per bootstrap replicate.

Results

The data matrix obtained from nr ITS sequences comprised 32 taxa and contained 663 sites. Among these, 384 (58 %) were constant, 128 (19 %) were variable but parsimony-uninformative and 151 (23 %) were parsimony-informative. The heuristic parsimony search generated 816 equally most parsimonious trees with a length of 558 steps, a consistency index (CI, excluding uninformative characters) of 0.4717, homoplasy index (HI, excluding uninformative characters) of 0.5283 and a retention index (RI) of 0.6687. The strict consensus tree of these 816 most parsimonious trees and bootstrap percentages (BP) for monophyletic groups is shown in Fig. 2.

In this analysis, the ingroup containing all *Lilium* species and one *Nomocharis* species is not monophyletic. North American species of *L.* sect. *Pseudolirium (L. philadelphicum* and *L. canadense)* were not resolved and separated from the rest of *Lilium* species. *L. auratum* and *L. japonicum* as representatives of *L.* sect. *Archelirion* form a clade with a strong support (100 BP). *L. martagon* is sister to the clade composed of representatives of *L.* sect. *Daurolirion,* sect. *Sinomartagon,* sect. *Leucolirion* and of *L. bulbiferum* of *L.* sect. *Liriotypus*. The clade formed by *Nomocharis saluenensis* and *Lilium nepalense* is sister to all members of *L.* sect. *Liriotypus* apart from *L. bulbiferum,* but the support for this relationship is low (51 BP). NE Turkish-Caucasian (99 BP) and European-SE Mediterranean lilies (85 BP) of *L.* sect. *Liriotypus* are sisters with a strong support (95 BP). However, the clade formed by the two NE Turkish endemics *L. ciliatum* and *L. akkusianum* (93 BP) is sister to all other European lilies with a bootstrap percentage of 85.

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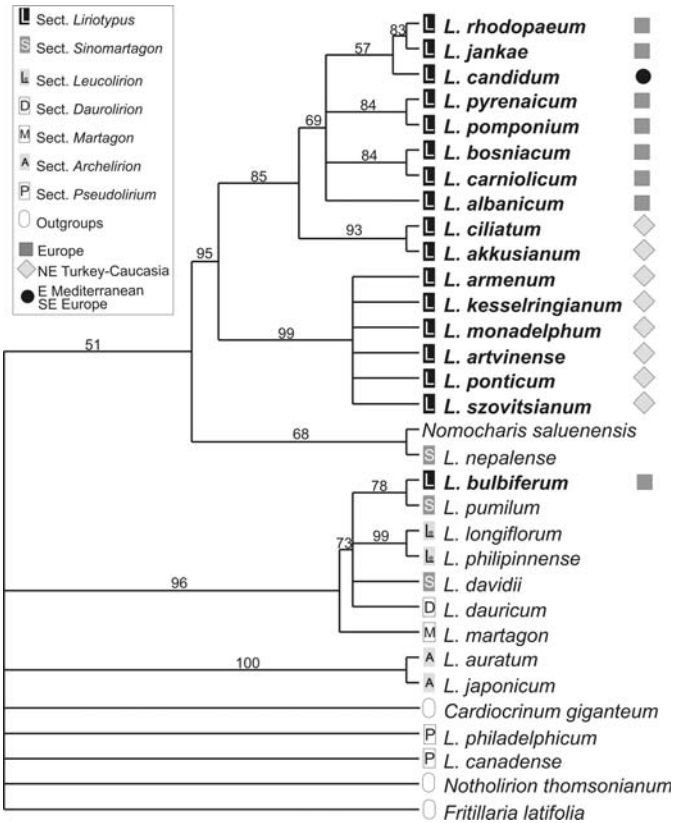


Fig. 2. Strict consensus tree of the 816 equally most parsimonious trees based on nrDNA ITS sequence information of the 32 taxa. – Percentages given above branches are bootstrap values (100 bootstrap replicates). The length of trees is 558 steps, the consistency index (CI, excluding uninformative characters) = 0.4717, the homoplasy index (HI, excluding uninformative characters) = 0.5283, the retention index (RI) = 0.6687. Sectional names of the genus *Lilium* are given according to the classification of Comber (1949).

Relationships among all other NE Turkish-Caucasian *Lilium* species *(L. armenum, L. kesselringianum, L. monadelphum, L. artvinense, L. ponticum* and *L. szovitsianum)* remain unresolved. *L. candidum* is sister to a clade of *L. rhodopaeum* and *L. jankae* (83 BP) with a low bootstrap support (57 BP).

Discussion

Systematics. – Based on the resulting phylogenetic trees, *Lilium* sect. *Liriotypus* is only monophyletic if *L. bulbiferum* is excluded. This confirms the corresponding results of Nishikawa & al. (1999) and Hayashi & Kawano (2000). Hence, the recent molecular studies, including our study, do not support Comber's (1949) classification of *L. bulbiferum* in *L.* sect. *Liriotypus*. Baker (1871) placed *L. bulbiferum* in *L.* sect. *Isolirion* with other erect-flowering lilies from North America and E Asia. Wilson (1925) and later Baranova (1988) followed Baker (1871) and placed *L. bulbiferum* in *L.* sect. *Pseudolirium,* which has the same species composition as Baker's (1871) *L.* sect. *Isolirion*. According to our results, *L. bulbiferum* is closely related to *L. pumilum* of *L.* sect. *Sinomartagon,* although the members of this section have Turk's cap flowers, and is otherwise placed in a clade comprising members also of *L.* sect. *Daurolirion,* sect. *Leucolirion* and

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sect. *Martagon.* In the study by Nishikawa & al. (1999) *L. bulbiferum* is placed in *L.* sect. *Daurolirion,* which is closely related to *L.* sect. *Sinomartagon.* The study by Hayashi & Kawano (2000) revealed a similar result. The position of *L. bulbiferum* is discussed in more detail in the following section in the light of morphological and other source of evidence.

Classification of the remaining species of *Lilium* sect. *Liriotypus* is less controversial apart from the question whether they should be included in *L.* sect. *Martagon,* as proposed by Baker (1871) and Wilson (1925), or in *L.* sect. *Liriotypus*. The type species of *L.* sect. *Martagon, L. martagon* differs from all other European lilies by having a verticillate leaf arrangement. In our phylogenetic trees this species is grouped with other E Asian species. Therefore, it is reasonable to conclude that the European lilies should be placed in *L.* sect. *Liriotypus,* not in sect. *Martagon*. Baranova's (1988) section *Eurolirium* (the type of the section name is *L. pyrenaicum*) includes all the species of section *Liriotypus* except *L. candidum,* which has trumpet shape flowers. Our results clearly indicate that *L. candidum* should not be classified in a unispecific section, because this would make the remaining group paraphyletic.

Morphological data and other evidence in conjunction with the phylogeny based on ITS. – In the group of species forming *Lilium* sect. *Liriotypus,* only *L. bulbiferum* has upward-facing flowers. Such a type of floral morphology is seen in other lilies from North America and Asia, where they have a scattered distribution. Because of their wide distribution range and little specialized flowers, Stoker (1939) considered these species as primitive. In contrast, Comber (1949) expressed the view that upward facing flowers appeared later as a result of parallel evolution in response to drier environmental conditions with less need to protect the pollen. Lighty (1968) proposed that *L. bulbiferum* shares common ancestors with the members of *L.* sect. *Sinomartagon* and *L. dauricum,* which gave rise to *L. bulbiferum* through a different evolutionary pathway, rather than with other European lilies. This last hypothesis gains support from our study, as well as the hypothesis does that the upward-facing flowers of *L. bulbiferum* are plesiomorphic in *Lilium.*

The other morphologically divergent species is *Lilium candidum* with snow-white, open funnel-shaped flowers, whereas the remainder of *L.* sect. *Liriotypus* has yellow or red Turk's cap flowers. The inner perianth segments of *L. candidum* are not papillose and are not distinctly hollow and with the stamens shorter than the style. Hibernating basal leaves arising from the bulb scales are seen only in *L. candidum,* which also has radical leaves. Lighty (1968) considers *L. candidum* as an evolutionary terminus of the route that prior gave rise to the other species of section *Liriotypus*.

Although their floral morphology is quite different from each other, the long-ciliate hairs on the leaf margins and on the flower buds of *Lilium ciliatum* and *L. akkusianum* are unique in the section. *L. ciliatum* has small, complete Turk's cap flowers, whereas *L. akkusianum* has large tepals, which are only slightly recurved at the tips. Karyological studies of *L. ciliatum* (Özdemir 2003) show $2n = 24$ acrocentric chromosomes, two pairs of which were shorter than the others. There are no chromosome data for *L. akkusianum,* which was recently described (Gämperle 1998).

The so-called *Lilium carniolicum* group includes *L. carniolicum, L. albanicum, L. bosniacum, L. jankae, L. pomponium* and *L. rhodopaeum*. In this group *L. albanicum, L. bosniacum* and *L. jankae* are often classified as subspecies or varieties of *L. carniolicum,* which is assumed to be a polymorphic species because of its variable flower colour and leaf indumentum (Grey-Wilson 1982, Stoker 1938). In this group *L. jankae* has yellow, strongly reflexed Turk's cap flowers and evenly pubescent veins on the ventral leaf face (Synge 1980). *L. bosniacum* has yellow or orange Turk's cap flowers with the leaf indumentum varying from glabrous to hairy. *L. albanicum* has yellow Turk's cap flowers and glabrous leaves and *L. carniolicum* has orange-red Turk's cap flowers with hairs on the on the ventral leaf face (Turrill 1953). A distinct species, *L. rhodopaeum,* is intermediate between *L. carniolicum* and the *L. monadelphum-szovitsianum* group (Synge 1980) and has lemon-yellow flowers with barely reflexed tepals. *L. pyrenaicum* has small, yellow Turk's cap flowers, *L. pomponium* has bright red Turk's cap flowers with papillose inner perianth segments.

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According to our phylogenetic analysis, the *Lilium carniolicum* group is polyphyletic. Matthews (1984) classified *L. pyrenaicum, L. carniolicum* (including *L. albanicum, L. bosniacum* and *L. jankae*) and *L. ponticum* (including *L. artvinense*) as subspecies of *L. pyrenaicum* based on the morphological similarities of the flowers and leaves. Nevertheless, our results do not support this hypothesis, because at least *L. ponticum* (including *L. artvinense*) is distinctly separated from the *L. carniolicum* group and also from *L. pyrenaicum*. In the light of their studies on genome size and chromosome organisation of *L. pyrenaicum, L. pomponium* and *L. carniolicum,* Siljak-Yakovlev & al. (2003) also suggest retaining them as separate species.

The Caucasian *Lilium monadelphum* group, which includes *L. armenum, L. szovitsianum, L. monadelphum* and *L. kesselringianum,* has similar large, yellow to pale cream flowers with tepals only slightly recurved, leaving the tube visible. Although Stewart (1947) examined 48 species and varieties of *Lilium,* only *L. monadelphum* of this group was included in his study. He found that *L. monadelphum* has a distinct karyotype with secondary constrictions on the long arms of the sixth chromosome pair.

The other Caucasian lilies, *Lilium ponticum* and *L. artvinense,* have smaller, deep orange to yellow flowers with strongly recoiled tepals hiding the tube (Davis & Henderson 1970). We consider *L. ponticum* and *L. artvinense* as conspecific, judging from their similar morphological characters and overlapping distribution.

Biogeography. – The Himalayas are considered to be the centre of origin of the genus *Lilium* with species having spread into the rest of Eurasia and North America (Patterson & Givnish 2002). According to Lighty (1968), *L. martagon* was the first species colonising Europe, a second migration from the north gave rise to the Central and SW European *L. bulbiferum* and the third route from the south through Caucasia into Europe resulted in all other European *Lilium* species. According to our phylogenetic analysis, *L. bulbiferum* was not descended from the same ancestor as the other European lilies, supporting the above hypothesis of a evolutionary origin of *L. bulbiferum* separate from the other European lilies.

Lilium pomponium and *L. pyrenaicum* clustered together in the phylogenetic tree. *L. pyrenaicum* occurs only in the eastern Pyrenees in both N Spain and in S France, growing in woods and mountain meadows (Matthews 1980). *L. pomponium* occurs on rocky hillsides in the Maritime Alps in S France and in NW Italy (Matthews 1984). They occupy the western limit of European lilies and there is a distance of more than a thousand miles between the areas of these two species and the rest of the *L. carniolicum* group.

Within the *L. carniolicum* group, *L. bosniacum* is distributed only in Bosnia and Herzegovina. *L. albanicum* has the southernmost distribution of them, occurring in the mountains of Albania, Y.R.P. Makedonia, Montenegro and N Greece. *L. rhodopaeum* occurs only in the Rhodope Mts in N Greece and S Bulgaria where it grows in alpine meadows and on rocky slopes (Matthews 1980). *L. jankae* is distributed in E Yugoslavia, Romania and Bulgaria (Matthews 1980).

Lilium candidum, which occurs naturally in SE Europe, Syria, Lebanon and Israel (Synge 1980), clusters together with other Balkan lilies. This is congruent with Lighty's (1968) view that a Balkan line gave rise to *L. candidum*.

According to Mandenova (1940), orographical and geographical isolation in the Caucasus played a critical role in the evolution of new *Lilium* species. Except for *L. martagon,* all of the *Lilium* species occurring in the NE Turkey-Caucasus region are confined to just this region. Only *L. monadelphum* extends to the northern side of Caucasus (Synge 1980), all the other species are dispersed in Georgia, Armenia and NE Turkey. Our results show that two lineages are present in this region, one giving rise to *L. ciliatum* and *L. akkusianum,* which are endemic to NE Turkey, and the other lineage to all Caucasian lilies.

Conclusion. – Our phylogenetic analysis obtained from the sequence analysis of the nuclear ribosomal ITS region shows that *Lilium* sect. *Liriotypus* is a monophyletic group if *L. bulbiferum*

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is excluded. Our results support the hypothesis that European species derived from three different routes; first *L. martagon* colonized Europe, a second route gave rise to *L. bulbiferum* and the last route gave rise to all other European *Lilium* species including *L. candidum*. Our analysis also shows that *L. ponticum* (incl. *L. artvinense*) is no part of the *L. carniolicum* group.

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