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Taxonomy and Phylogeny of the Genus *Lilium*

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ABSTRACT

Lilies have a long history as ornamental plants. Today, there is an ever increasing variety of new lily cultivars due to the significant progress in the propagation and development of new methods in breeding. The domesticated native species have retained their place along with new hybrids in commercialized horticultural industry, and they have sustained their invaluable potential for the breeding of new cultivars for garden use as well as for greenhouse culture. Systematics has always played an important role in plant breeding, giving guidelines for hybridization, although biotechnology has introduced new solutions for many problems that were evolutionary obstacles especially in inter-specific crossings before. The genus *Lilium* has been a subject of variable suggestions for classification systems, and the process still continues. The currently accepted concept for the phylogenetic and taxonomic system for all species is based on geographical, structural and genetic information. In our review, we give an insight into the latest progress in revealing the taxonomical relationships within the genus. According to the existing GenBank sequence data, we have constructed a phylogenetic tree consisting of the main species and sections of the genus. Provided with species photos, the tree gives a brief overview of phylogeny- and morphology-based classifications, which are not always congruent. In the tree mainly all species grouped into sections defined within the genus, but *L. bulbiferum* and *L. dauricum* grouped equally with the species in *Sinomartagon* and not with each other. Even though these two species share many morphological features, the phylogenetic tree questions the existence of the section *Daurolirion* and potentially gives a blueprint for classification in the future.

Keywords: bulbous plants, classification, lilies, systematics

CONTENTS

INTRODUCTION

Lilies (genus *Lilium* L.) have retained their position as one of the most important ornamental plant group both as garden plants as well as pot cultured and cut flowers. The genus consists of approximately 100 species that range from the Sierra Nevada and Rocky Mountains to eastern North America through Europe and Middle East to the Caucasus Mountains, Siberia, and Eastern Asia. The only continents devoid of endemic *Lilium* species are Africa, South America, Australasia and Antarctica (Woodcock and Stearn 1950; Rockwell *et al.* 1961).

The natural distribution of the genus suggests that the main speciation occurred after the separation of the land masses that later formed the present continents of Eurasia and North America, and that the main species groups of the early classification were established mainly based on the geographic isolation. Although the structural differences as well as the geographical distribution of the species are variable, the genome structure on karyotype level is surprisingly constant throughout the genus. Haploid chromosome number is invariably 12, and polyploidy is almost non-existent in natural populations. Although there are differences in the genome sizes between species, the common feature is the exceptional size, ranging from 32 to 100 billion bp (Bennett and Smith 1976; Sentry and Smyth 1989; Siljak-Yakovlev *et al.* 2003). In record, the oldest garden plant is *L. candidum* L., which has been grown for its decorative value since the ancient times in Egypt and Middle East. Lilies have had their place also in European

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Invited Review

gardens for centuries. For example, various strains of *L. bulbiferum* L. are found all around the countryside in Nordic countries, as far as Lapland, i.e. the Northern parts of Finland, Norway and Sweden (Pelkonen *et al.* 2007). Originally these lilies came from Central Europe where they had been grown in gardens since the 16th century (Bos 1993). Similar to *L. bulbiferum*, *L. martagon* L. and *L. lancifolium* Thunb. (syn. *L. tigrinum* Ker Gawl.). are also old garden plants. Along with these common species, there are some examples of other, less known garden lilies of old origin, e.g. *L.* × 'Marhan' and *L.* × 'Testaceum' Lindl.

TRADITIONAL OR HISTORICAL CLASSIFICATION

Since the very start of cultivation, lilies have been classified according to different traits: the earliest classifications were typically based on the form, size and colour of the inflorescence. The first cultivated species were native to Southern and Middle Europe, from where they were adopted to gardens. Many of these domesticated natural species have horticultural importance still today, and are commercially available with the modern hybrids and cultivars.

Along with the introduction of new species from Eastern Asia and Northern America to Europe, the geographical origin was combined with the morphological classification criteria by the first taxonomists that were mainly European. Furthermore, the first attempts to hybridize intersectional species were not successful, which confirmed the perception that the species groups were stable, and that cross-breeding between groups was impossible. Arising new cultivars were mainly hybrids of related species within the same group, e.g. Mid-Century hybrids (Asiatic group), Marhan hybrids (*Martagon* group) or Olympic hybrids (Trumpet group) (Woodcock and Stearn 1950; de Graaff 1952; Rockwell *et al.* 1961, de Graaff and Hyams 1967).

New pollination techniques, though, finally broke the obstacle of the intersectional incompatibility, and a new generation of interspecific hybrids, like LA (*L. longiflorum* Thunb.× Asiatic) and OT or Orienpet (Oriental × Trumpet) were introduced to global markets. This proved that the apparent crossing barrier was merely structural or temporal, as the interspecific hybrids were also capable of producing fertile offspring.

HISTORY AND THE CRITERIA OF CLASSIFICATION

Relationships between the closely related genera of lily-like bulbous plants (*Liliae*) have been studied from the beginning of the classification work, and some agreement in the order of the genera has been established, as described below. In the beginning, the definitions between the closely related genera were not clear, and several species that were originally included in *Lilium* were later transferred to different genera. Baker (1879) included the subgroup *Cardiocrinum* (Endl.) Lindl. into *Lilium*, which later was again separated into its own genus (Wallace 1879). On the other hand, some species originally classified in the genus *Nomocharis* Franch. were returned back to the genus *Lilium* and now form the subgroup *Lophophorum* in the *Sinomartagon* group. Buxbaum (1937) arranged the six genera (*Fritillaria* excl. *Korolkowia* Regel, *Notholirion* Wall. ex. Boiss., *Cardiocrinum*, *Nomocharis* and *Lilium*), based on the morphological features, in a suggestive evolutionary tree. In the tree, *Nomocharis* and *Lilium* formed their own lines, which arose from the main branch comprised by the rest of the genera (reviewed by Woodcock and Stearn 1950). Although this tree was more of a speculative model, it definitely created a layout for more precise molecular analyses of evolutionary relationships within the genera (Patterson and Givnish 2002; Leitch *et al.* 2007).

The main morphological criteria used for taxonomic classification and as the grounds for the evolutionary relations were the structure, position and colour of inflorescence, the arrangement of the leaves, the form and structure of the bulb, the ability to produce bulbils and the type of germination. The species were divided according to the flower form and pose into three main subgroups: *Leucolirion*, *Martagon* and *Dauricum* (Woodcock and Stearn 1950).

Based on Baker (1875), the genus *Lilium* was divided into five subgroups, or defined at the time as subgenera, and *Cardiocrinum* was included as the first subgenus, but again soon excluded as a separate genus (Wallace 1879). The rest of the species known at the time were divided into the remaining four subgroups: *Eulirion* consisted of the present section of *Leucolirion* and species from the sections *Liriotypus*, *Sinomartagon* and *Pseudolirium* with funnel-shaped flowers. *Archelirion*, besides the species of the current section with the same name, comprised some species now classified in section *Sinomartagon* with broad petals. *Isolirion* consisted of the current section *Daurolirion* and all other species with erect flowers. Finally *Martagon*, consisting of all species with strongly recurved petals including the species currently present in this section, and most of the species from the current sections *Liriotypus*, *Pseudolirium* and *Sinomartagon*.

As can be concluded from the text above, major reorganizations have taken place since the early days, and various classification models have been presented. The need for re-organization of the genus arose from the introduction of new species, mostly from Eastern Asia, and Northern America (Wilson 1925). In addition, horticultural use and the introduction of a new generation of cultivars required an update of the classification. Various authors created several new systems and categories, where even some influence of different scientific tradition can be recognized.

Woodcock and Stearn (1950) based their classification partly on the previous system described by Baker, but they had already excluded *Cardiocrinum* and some species of *Nomocharis* and *Fritillaria* Tourn. ex L. from *Lilium*. Their grouping was merely based on the structure, although they also had some emphasis on the geographical aspect. They, for instance, divided the American species into two geographical groups: Eastern, or Atlantic coast and central lilies, and Western, or Pacific coast lilies. The main groups presented by Woodcock and Stearn (1950) were: *Leucolirion*, identical to the present one except for the inclusion of some American species with funnel-shaped flowers; *Archelirion* with *L. auratum* Lindl., *L. speciosum* Thunb. and *L. lancifolium*; *Pseudolirium*, corresponding to the Baker's *Isolirion*; and *Martagon* identical with Baker's system (Woodcock and Stearn 1950).

Baranova (1990) introduced a system with the greatest number of sections. In addition to the sections *Martagon*, *Archelirion*, *Sinomartagon* and *Pseudolirium*, she added also *Eurolirion*, which is identical with the current section *Liriotypus*, *Pseudomartagon* identical to the current section *Pseudolirium* (American species), *Regalia* identical to the current section of *Leucolirion*, *Sinolirion* having *L. concolor* var. *pulchellum* (Fisch.) Baker as the only species, *Pseudolirium* with all erect-flowered species, *Nepalensia* including species of the Southern Himalaya, and *Lophophora* with species forming their own group in *Sinomartagon* (former *Nomocharis* species).

The core of the former classification models remained in the more precise system by Comber (1949) with some modifications used in horticulture (reviewed by Mc Rae 1998). In this system, which is now widely accepted, structure and biology of the species were considered, besides geography, as the classification criteria. Seven groups were formed, partly adopted from the former systems with slight adjustments and re-arrangement of the subgroups: *Martagon*, *Pseudolirium*, *Liriotypus*, *Archelirion*, *Sinomartagon*, *Leucolirion* and *Daurolirion*.

GENOME SIZE AND ORGANIZATION OF LILY SPECIES

The genomes of lilies have been under investigation for decades, and have provided further criteria for classification of some species (e.g. see Siljak-Yakovlev *et al.* 2003). The first published reports of karyotype analysis revealed the special features of this genus: large size and constant number of the chromosomes (Abraham 1939; Stewart *et al.* 1943; Stewart 1947; Sharma *et al.* 1957). An exceptional feature of the lily genome is its immense size. For example, the genomic DNA of *L. henryi* Baker consists of 32 billion base pairs, and in some species it can be as high as 100 billion bp (Bennett and Smith 1976; Sentry and Smyth 1989). In comparison, the corresponding numbers of other scientifically or economically important plants are: *Arabidopsis* 0.3, *Allium ca*. 31, *Asparagus* 2.6, *Petunia* 2.6, rice <1 and tulip 50-60 billion bp (Arumuganathan and Earle 1991). Human DNA consists of 2.9 billion bp (IHGCS 2004). The lily genome is organized in large metacentric and subtelocentric chromosomes. The haploid number of chromosomes is 12, and it is very constant throughout the genus. Natural species are mostly diploid (2n = 24), but some species have triploid forms (3n = 36) that are sterile. Some naturally occurring tetraploids have also been found, and these are usually fertile. It has been assumed that interference in the meiosis of reproductive cells can result in spontaneous formation of polyploidy in lilies. There are also examples of spontaneous inter-specific hybrids that show a normal diploid chromosome number. Abnormal chromosome numbers have been found in some studies, but in general, they are very rare (Abraham 1939; Stewart and Bamford 1943; Stewart 1947; Sharma and Bhattacharyya 1957; Siljak-Yakovlev *et al.* 2003).

As the breeding techniques have been developed, the production of tetraploid plants has been accomplished artificially by treating either seeds or bulb scales of diploid plants with colchicine or oryzalin. Tetraploid forms have proved more robust in their growth habit, having a thicker texture of tissues and higher resistance to diseases (Emsweller 1949; Lim and Van Tuyl 2007; Balode 2008). When crossed with diploid forms they produce triploid offspring (McRae 1998). However, generally the normal diploid chromosome number predominates both within species and within hybrids or cultivars. The immense size of the lily genome is partly due to the substantial amount of repetitive sequences in the chromosomes. Considering that the genome organization of lilies is highly conserved, and that the repetitive sequences have remained in the genome through millions of years of evolution, their importance to lily species must have evolutionary significance.

FROM STRUCTURE TO GENOME AND CLASSIFICATION

The previously mentioned historical classes have remained as the basis for more accurate and precise classification derived from the genomic structure of species and strains. However, classification based only on the morphological characteristics has not always been straightforward in the *Lilium* genus. This is because even distantly related species can share similar characteristics such as flower shape (**Fig. 1**; Mitchell 1998). An example of this is *L. henryi* that was classified by Comber as a species belonging to *Sinomartagon* linked to section *Archelirion*. Later on, *L. henryi* was classified into the section *Leucolirion* based on seed fertility (Leslie 1982).

In general, classification within the genus *Lilium* has caused debate and the systems for taxonomy have changed several times (Hayashi and Kawano 2000). For these reasons, genetic marker-based methods for classification of *Lilium* species have been brought into play. Generally, one of the earliest methods used is allozyme-based analysis, followed by random amplified polymorphic DNA (RAPD), and terminal restriction fragment length polymorphism (T-RFLP) that take advantage of the large genome size with numerous mismatches in the genomic DNA. Most recently, several sequence-based markers such as ITS and either non-coding or coding regions of the chloroplast DNA have been used to classify species and strains of the *Lilium* genus. In the following section, we give examples and review the use of these methods and their influence on the taxonomy in *Lilium*.

MOLECULAR EMPHASIS IN TAXONOMIC STUDIES

The allozyme-based analysis has not provided substantial new information on the taxonomy of lilies, but it has successfully been used to study the biogeography of two bulbous species, *L. longiflorum* and *L. formosanum* Wallace that are endemic to the subtropical archipelago located in East Asia (Hiramatsu *et al.* 2001). In that study, 13 isozyme loci were analyzed and the allozymes had significantly high variability and divergence in the insular endemic species *L. longiflorum*. However, *L. formosanum* had less variability and divergence in the allozyme pattern and was genetically close to the southern peripheral populations of *L. longiflorum* (Hiramatsu *et al.* 2001). Together with other biological and insular geohistorical information, results could be drawn that pointed to origin of *L. longiflorum* from the continuous part of the ancient Asian continent from the end of the Pliocene, whereas *L. formosanum* presumably originated from southern populations of *L. longiflorum* of the mainland of Taiwan that was separated around the late Pleistocene (Hiramatsu *et al.* 2001).

In general, RAPD analysis has widely been used for studying genetic variation in a range of cultivated plants (Matsumoto and Fukui 1996; Wallner *et al.* 1996; Galderisi *et al.* 1999; Friesen and Blattner 2000; Palombi and Damiano 2002). While RAPD is often criticized for having insufficient capacity for resolution of genetic variations, RAPDs have proven very useful for the study of genetic diversity in *Lilium* (Yamagishi 1995; Persson *et al.* 1998; Wen and Hsiao 2001; Pelkonen *et al.* 2007; İkinci and Oberprieler 2010). RAPD is a multilocus method, providing phylogenetic information contained in several loci, and the method is easier and more affordable to use than the typical single-locus, sequence-based methods.

Yamagishi (1995) analyzed 76 primers and identified 18 RAPD markers that were useful for fingerprinting in 13 species of *Lilium*, including *L. lancifolium*, *L. leichtlinii* Hook.f., *L. concolor* Salisb., *L. candidum*, *L.* × 'Formolongi' cv. 'Hakuho', *L. longiflorum*, *L. formosanum*, *L. regale* E.H.Wilson, *L. henryi*, *L. speciosum*, *L. auratum*, *L. rubellum* Baker, *L. japonicum* Thunb. and *L. nobilissimum* Thunb. The markers developed by Yamagishi (1995) and 20 additional primers were further tested and used for phylogenetic analysis of strains of *L. bulbiferum* by Pelkonen *et al.* (2007). The strains were compared with species of a close taxonomic group *Dauricum* (*L. dauricum* Ker-Gawl., syn. *L. pensylvanicum* Ker.-Gawl. and *L. maculatum* Thunb.), because some cultivated strains of *L. bulbiferum* morphologically resemble these species. According to the phylogenetic analysis, the strains were divided into four groups. The results showed an interesting division of the cultivated strains in two subgroups where the trait to form bulbils was characteristic to subgroup I. In the phylogenetic, tree the cultivated strains differed from each other as much as from the seedling strains, which reflects high variation caused by genetic isolation (Pelkonen *et al.* 2007). The results also confirmed that the studied cultivars were natural forms of *L. bulbiferum* species (Pelkonen *et al.* 2007).

Recently, RAPD was employed for studying the species boundaries in six closely related *Lilium* species of the section *Liriotypus*; *L. ciliatum* P.H.Davis, *L. akkusianum* Gämberle, *L. ponticum* K.Koch, *L. kesselringianum* Miscz., *L. armenum* (Miscz. ex Grossh.) Manden. and *L. szovitsianum* Fisch. & Avé-Lall. by İkinci and Oberprieler (2010). They analyzed 108 primers, yielding 11 markers for RAPD

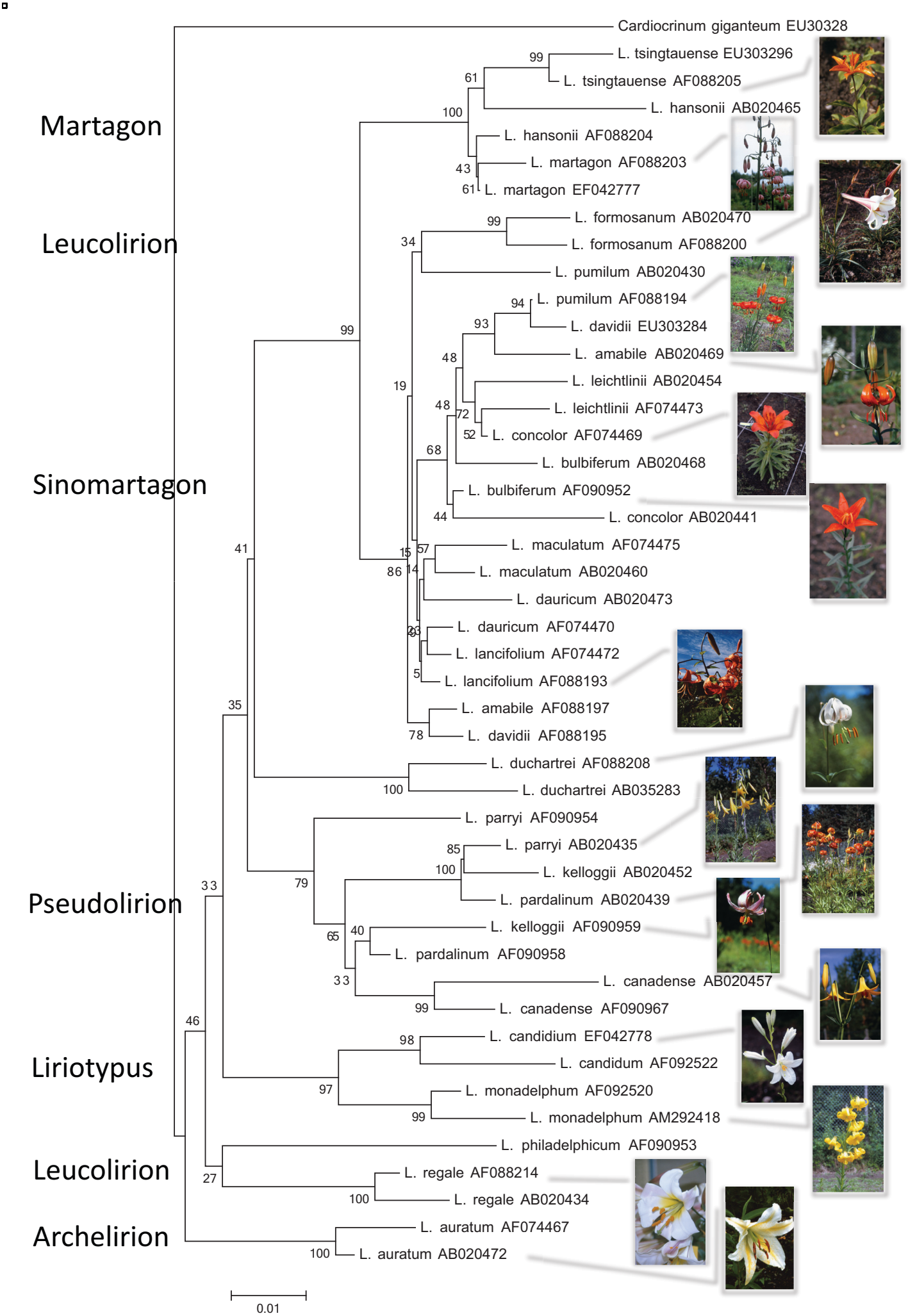
Cardiocrinum giganteum EU30328
L. tsingtauense EU303296
L. tsingtauense AF088205
L. hansonii AB020465
L. hansonii AF088204
L. martagon AF088203
L. martagon EF042777
L. formosanum AB020470
L. formosanum AF088200
L. pumilum AB020430
L. pumilum AF088194
L. davidii EU303284
L. amabile AB020469
L. leichtlinii AB020454
L. leichtlinii AF074473
L. concolor AF074469
L. bulbiferum AB020468
L. bulbiferum AF090952
L. concolor AB020441
L. maculatum AF074475
L. maculatum AB020460
L. dauricum AB020473
L. dauricum AF074470
L. lancifolium AF074472
L. lancifolium AF088193
L. amabile AF088197
L. davidii AF088195
L. duchartrei AF088208
L. duchartrei AB035283
L. parryi AF090954
L. parryi AB020435
L. kelloggii AB020452
L. pardalinum AB020439
L. kelloggii AF090959
L. pardalinum AF090958
L. canadense AB020457
L. canadense AF090967
L. candidium EF042778
L. candidum AF092522
L. monadelphum AF092520
L. monadelphum AM292418
L. philadelphicum AF090953
L. regale AF088214
L. regale AB020434
L. auratum AF074467
L. auratum AB020472
Martagon
Leucolirion
Sinomartagon
Pseudolirion
Liriotypus
Leucolirion
Archelirion
0.01

Fig. 1 (Previous page) A phylogenetic tree of the genus *Lilium* based on ITS sequences published mainly by Nishikawa (1999) and Dubouzet and Shinoda (1999), together with species photographs and taxonomic classification. To build the tree, we used the Neighbour-joining method on Molecular Evolutionary Genetics Analysis (MEGA) (Tamura *et al*. 2007). The robustness of the phylogeny was tested by bootstrap analysis using 1000 iterations. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). Photo credits: Veli-Pekka Pelkonen.

fingerprinting, and performed neighbour-joining cluster analysis based on the RAPD analysis. In the resulting phylogenetic tree, *L. ciliatum* and *L. akkusianum* were clearly separated from the rest four species. An analysis of molecular variance (AMOVA) indicated a weak genetic differentiation within the species (İkinci and Oberprieler 2010). Similar results were found by İkinci (2010) in the RAPD-based analyses on *L. albanicum* Griseb. and *L. chalcedonicum* L.

The RFLP method is rarely used for phylogeny studies on *Lilium*. There is one such report where Haruki *et al.* (1997) successfully used PCR-RFLP of the ribulose-1,5-bisphosphate carboxylase large subunit (*rbcL*) and nuclear ribosomal DNA regions for phylogenetic analysis of nine *Lilium* species belonging to sections *Archelirion* and *Sinomartagon*. Sequencing of the chloroplast and ribosomal DNA regions has been applied most often for phylogeny studies in the genus *Lilium*. Already in 1999, Nishikawa *et al.* used sequences of the internal transcribed spacer (ITS) regions of the ribosomal DNA to evaluate phylogenetic relations over the entire genus. The results from that study and suggestions on the classification in the genus are discussed further in this review. Another study by Dubouzet and Shinoda (1999) on 16 *Lilium* species and one variety in Japan, done internal transcribed sequencing (ITS), supported mainly the validity of Comber's classification system with minor suggestions for modifications. ITS sequencing has also been used to study phylogeny of the species group associated with *Lilium carniolicum* Bernh., which consists of several taxonomically ambiguous taxa endemic to the European flora (Rešetnik *et al.* 2007). Phylogenetic trees showed that all taxa in the group were very closely related, as the group was monophyletic. The analyses suggested that *L. chalcedonicum* is more closely related to *L. carniolicum* than previously thought and that *L. albanicum* and *L. jankae* A.Kern. are distinct from *L. carniolicum* (Rešetnik *et al.* 2007).

Sequences in the three spacers of the chloroplast DNA, *trnT-trnL*, *trnL-trnF* and *atpB-rbcL* are another variable region that has been used for phylogeny analysis of *Lilium*. Nishikawa *et al.* (2002) used these chloroplast sequences for the analysis of the section *Archelirion*, which was divided into two major clades. One clade consisted of *L. auratum* (var. *auratum*) and *L. rubellum*, and the other clade had the remaining taxa analyzed, which all were monophyletic (Nishikawa *et al.* 2002). Recently, the coding regions of the *rbcL* and *matK* genes of the chloroplast DNA were used for the phylogenetic classification of the entire family *Liliaceae* (Muratovíc *et al.* 2010). The analysis produced results that were in accordance with the taxonomic concept of *Liliaceae* proposed by Tamura (1998). The authors stated that the *matK* gene has better phylogenetic resolution than the *rbcL* gene, which has evolved more slowly. According to the phylogenetic analyses, the genus *Lilium* has three major clades where *Nomocharis pardanthina* Franch. and *Nomocharis saluenensis* Balf.f. were ingroup taxa of *Lilium*. The analyses demonstrated further that *Notholirion* Wall. ex Boiss., *Cardiocrinum*, and *Fritillaria* are sister groups of *Lilium* (Muratovíc *et al*. 2010). The suggested changes due to the molecular sequence-based phylogenies of *Lilium* are discussed in more detail below.

NEW ASPECTS ON THE CLASSIFICATION OF *LILIUM*

Phylogenetic analyses have caused rearrangements in the classification of *Lilium*. In the ITS-sequence based phylogenetic tree presented by Nishikawa *et al*. (1999), most of the species classified by morphology were clustered separately, each in their own clades at the section level. Nishikawa *et al.* (1999) further demonstrated based on the ITS phylogeny that the section *Daurolirion* is not independent of *Sinomartagon*, and that the two sections could be integrated as *Sinomartagon*. Further they suggested *L. henryi* and *L. bulbiferum* to be classified into subsection 6a and *Sinomartagon–Daurolirion*, respectively, and that subsection 6b was more closely related to *Sinomartagon* than subsection 6a, *Archelirion* arising directly from *Sinomartagon*. Finally, Nishikawa *et al.* (1999) stated that *Lilium* is much closer to *Nomocharis* than *Cardiocrinum*. Dubouzet and Shinoda (1999) also analyzed Japanese *Lilium* species by ITS sequencing with less modification to the Comber's classification system, generating a phylogenetic tree that was supported by classification based on crossing experiments. As a result of their analysis, they suggested to transfer *L. dauricum* (syn. *L. pensylvanicum*) to the section *Sinomartagon*.

A thorough phylogenetic analysis based on chloroplast and ITS sequences was recently made on European lilies (Muratovíc *et al.* 2010). Together with earlier ITS- and RAPD-based studies, though not always straightforward, the results indicated that European lilies could be divided into three groups, that of *L. martagon*, being closely related to East Asiatic lilies and *L. cattaniae* (Vis.) Vis. (syn. *L. martagon var. martagon*), the *L. bulbiferum* group that was distinct from the other European lilies having an ambiguous placement in the phylogenetic trees, and the group of remaining species that belonged to Comber's *Liriotypus* subsections 3a, 3b and 3c. Muratovíc *et al.* (2010) stated that *L. cattaniae* and endemic taxa such as the *L. carniolicum* complex including *L. albanicum*, *L. bosniacum* (Beck) Fritsch, *L. carniolicum*, and *L. jankae* still had a questionable taxonomical status. For example, earlier ITS analyses had clustered *L. albanicum* and *L. jankae* close but not together with *L. carniolicum*, but these analyses had failed to differentiate *L. bosniacum* from *L. carniolicum*, and as a result, *L. bosniacum* was not considered a species (Rešetnik *et al*. 2007). However, fluorescence *in situ* hybridization (FISH), chromomycin and DAPI staining analyses had clearly differentiated these species (Muratovíc *et al.* 2005; Siljak-Yakovlev *et al.* 2003).

The phylogenetic analyses based on ITS and rpS4-trnT-trnL sequences and genome size assessments on several European lilies populations representing 10 species belonging to section *Liriotypus* and section *Martagon* revealed distinct *Lilium* sections, whereas at the subsection level, a remarkably low genetic differentiation was observed (Muratovíc *et al.* 2010). For example, the group consisting of the European species of *Liriotypus* section had an extremely low genetic differentiation with variable positions, exhibiting mainly non-fixed polymorphism. In that study, also significant variation at geographic and ecological level was detected within several species (Muratovíc *et al.* 2010). As a result, Muratovíc *et al.* (2010) stated that taxonomic misunderstandings are not always solvable by molecular phylogeny, especially if one specimen is used as the representative of the species.

On-going research with constantly improving methods bring new and more accurate information concerning the variability within given species, and the position of species in the genus of *Lilium* (Guo *et al.* 2011; Zhi *et al.* 2011). In addition, some results of recent studies are suggesting, that there is even ground for the reunion of the genera *Nomocharis* and *Lilium* (Gao *et al.* 2012a, 2012b). Using different approaches it is also possible to evaluate the relevance of criteria and methods used in revealing the environmental

factors that affect the processes of genetic differentiation, and subsequent emergence of new species (Douglas *et al.* 2011; Sommers *et al.* 2011).

Here, we performed a phylogenetic analysis on the published ITS sequences (mainly by Nishikawa *et al.* 1999; Dubouzet and Shinoda 1999) of *Lilium* (**Fig. 1**). In the tree, the six sections of the genus, *Liriotypus*, *Martagon*, *Sinomartagon*, *Pseudolirium*, *Leucolirion* and *Archelirion* were mainly clustered into their own subgroups, except for *Leucolirion* where *L. regale* and *L. formosanum* were separated and formed their own monophyletic groups (**Fig. 1**). However, there is too low bootstrap value to support this separation. Another exception was *L. philadelphicum* L., which formed a monophyletic group and was more closely related to *L. regale* than any of the species in the *Pseudolirium* group, but this could not be supported by a high bootstrap value either. The strains within a species mainly clustered together, but not always. For example, one *L. pumilum* Delile in P.J.Redouté strain clustered with *L. formosanum* and the other was quite distant, clustering with *L. davidii* Duch. ex Elwes. *L. bulbiferum* and *L. dauricum* formed monophyletic groups within the group and did not cluster together, but clustered with high bootstrap values with other species in the *Sinomartagon* group, such as *L. maculatum*, *L. concolor* and *L. leichtlinii* (**Fig. 1**). The phylogenetic tree was combined with species photos, which nicely demonstrates the interface between morphology (flower shape) and phylogeny, and that it is not congruent by default. The separation of *L. bulbiferum* and *L. dauricum* that share many morphological features is a good example of this in the tree (**Fig. 1**).

To summarize, although the constant accumulation of new information brings more understanding and clarity to the previous concept, the basic classification has proved to be a solid foundation with some adjustments. As is shown above, some species groups have remained to some extent disputable, or even controversial, and more information is needed to achieve agreement on their status. *E.g.* the species from the Eastern Mediterranean region and Caucasus area (*L. chalcedonicum*, *L. kesselringianum*, *L. monadelphum*, *L. szovitsianum*, etc.) have features by which they are combined to a species as a subspecies or variation in some studies, and regarded as separate species in others. The current understanding of the taxonomy of *Lilium* is discussed below.

Martagon

Martagon group is unique in a sense that it does not have any close relatives within the *Liriotypus Sinomartagon* sections, although it has resemblance with species of both sections. It represents a sort of connecting clade between these two subgroups of the genus. Still, in Comber's (1949) classification, *Martagon* subgroup was placed into *Sinomartagon* mainly due to its geographical distribution. Newer molecular data supports this position.

The most variable species of the genus is *L. martagon s.l.*, which also has the most widespread natural distribution, ranging from Central Europe up to the Eastern parts of Siberia (Persson *et al.* 1998). Persson *et al.* (1998) demonstrated by RAPD analysis that domesticated cultivars of *L. martagon* have no significant genetic differences from the genuine species, as in their study, the domesticated strains fell within the variation of the species. More generally, *L. martagon* can be considered as a group of closely related geographical forms or variations of the same species. The only exception is the entirely white form (syn. *L. martagon* var. *album* Weston; homonyme *L. martagon* f. *album* (Weston) Beck) which seems to be only an albino form originating from a simple secreting mutation, or an ultimate light form of the colour scale of the existing forms, as is the case in many other plant species. White forms and albinos are found also in some other lily species. In *L. regale*, for instance, the white form has totally white petals without the typical purple epidermis of the exterior of the petals, or without the yellow base of the interior of the perianth segments that form the characteristic throat of the type form flowers. In some other species, on the other hand, the possible albinism cannot be observed due to the total lack of pigmentation. This kind of species is *L. candidum*, which has not been reported to show any colour forms.

Also a couple of other species are included in the *Martagon* group that can be distinguished from the actual *L. martagon* clade and from the section of *Martagon*. These are *L. hansonii* Leichtlin ex D.D.T.Moore, *L. medeoloides* A.Gray, and *L. tsingtauense* Gilg, which have many common features with the species *L. martagon*. The leaf orientation is whorled, and the flowers are borne in racemes. Unlike *L. martagon* s.l. and *L. hansonii*, the flowers of *L. tsingtauense* are posed upwards, although the form of the perianth segments and the sexual parts of the flowers have a great resemblance with the other *Martagon* group species. Originating from a restricted area in Korean peninsula, *L. hansonii* is less variable both in its geographical distribution as well as in its phenotype than *L. martagon*. As a proof of their evolutionary syntax, their interspecific crossing has proved easy, and in many cases also spontaneous. One of the earliest interspecific hybrids was L. × 'Marhan', which is a *grex* between various *L. martagon* forms and *L. hansonii*.

Pseudolirium (American group)

This subgroup is originally based on the geographical occurrence of the species. It consists of *ca*. 20 species typically having rhizomatous bulbs and whorled leaves. Most of the American species have delayed hypogeal germination with the exception of three species: *L. catesbai* Walter, *L. parryi* S.Watson, *L. parvum* Kellogg, and *L. philadelphicum*. Leaves are mostly arranged in whorls and the flower size, form, and pose vary from species to species.

In this group *L. canadense* L. has the widest natural distribution ranging through the whole continent from coast to coast. It is also the most northern species, as it can be found up to the southern parts of Canada. *L. canadense* is the first American species of the genus introduced to Europe, where it was first described by Linné. Another moderately widespread species within the American group is *L. pardalinum* Kellogg, which is more Southern and Western than *L. canadense*.

Most of the native American lilies originate from the South Western part of the continent. Two species are quite distinct from the other groups as is also shown in the phylogenetic tree of **Fig. 1**, namely *L. philadelphicum* and *L. catesbaei*. They are small species, which differ from the other American species by having upright flowers and immediate epigeal germination. *L. catesbaei* is also the only species in the group with alternate leaf orientation (Woodcock and Stearn 1950; Jefferson-Brown and Howland 1995).

Archelirion (Oriental group)

This group is formed by six Eastern Asian species that differ from the *Sinomartagon* group species by the morphology of inflorescence and foliage. Typically they have very large petals, bowl to open funnel shaped flowers and broad, scattered leaves with distinguishable stalk. All species in this group originate from the South Eastern parts of China, Korean peninsula and Japan, where some of the species are endemic. In the traditional classification, this group consisted of nine species indigenous to Japan and Eastern China including *L. henryi*, which was later placed to *Sinomartagon* (Nishikawa *et al.* 1999).

In Archelirion some adjustments have been suggested based on chloroplastic DNA sequence (Nishikawa *et al.* 2002). According to that study, the division of *Archelirion* group into two subgroups or clades could be established. Hence, the type form of *L. auratum* (var. *auratum*) along with *L. rubellum* formed the *Rubellum* type, and the form *L. auratum* var. *platyphyllum* Baker was placed to the Japoni-

cum group comprising the rest of the Archelirion species *L. japonicum*, *L. nobilissimum*, *L. speciosum* and *L. alexandrae* Coutts (syn. *L. longiflorum* var. *alexandrae* (Baker) E.H.Wilson).

Liriotypus (*Candidum* group)

The position of *L. candidum* is confusing, as in some analyses it is placed close to *L. ciliatum*, and in others closer to *L. chalcedonicum*, with which it also hybridizes (*L.* × 'Testaceum'). According to Nishikawa's (1999) results *L. candidum* and *L. ciliatum* did not have close relation within the *Liriotypus* group. This is also supported by the obvious morphological characteristics. Thus, *L. candidum* may be kept alone within *Liriotypus*, and *L. ciliatum* may be placed closer to the Caucasian species group, described by Muratovíc *et al.* (2010) as the subgroup 3b, consisting of *L. chalcedonicum*, *pyrenaicum* Gouan, *pomponium* L. and L. *carniolicum* incl. *jankae*, *albanicum*, *bosniacum* and *rhodopaeum* Delip. According to the results by Nishikawa *et al.* (2002), some adjustments could be applied to the system of Comber (1949): both molecular and morphological data demonstrated that *L. bulbiferum* could not be placed in the Eurasian *Liriotypus* section by merely geographical grounds. The data showed that similarities in morphological characteristics between *L. bulbiferum* and *Daurolirion* group supported its inclusion to this group. This is also in agreement with the recent observations by Muratovíc *et al.* (2010).

Sinomartagon (Asiatic group)

The largest group of the genus by the number and variation of the species is *Sinomartagon*, which consists of approximately 20 Chinese species. Common features in this group are scattered leaves, flowers with recurved petals and horizontal or drooping pose, and immediate hypogeal germination. They originate from a wide area ranging Eastern parts of Siberia to Central and Eastern China down to the Himalayas (Woodcock and Stearn 1950; Jefferson-Brown and Howland 1995). Because of the high diversity, the group has been divided into 3-4 subgroups depending on the classification. According to Comber (1949), with some adjustments by Nishikawa *et al.* (2002), the subgroups are: a) *L. davidii* (var. *willmottiae* (E.H.Wilson) Raffill), *L. duchartrei* Franch., *L. henryi*, *L. lancifolium*, *L. lankongense* Franch., *L. leichtlinii* (var. *maximowiczii* (Regel) Baker), b) *L. amabile* Palib., *L. callosum* Siebold & Zucc., *L. cernuum* Kom., *L. concolor* (*var. pulchellum*), *L. pumilum*, and c) *L. amoenum* E.H.Wilson ex Sealy, *L. henricii* Franch., *L. mackliniae* Sealy, *L. nanum* Klotzsch, *L. nepalense* D.Don, *L. oxypetalum* (D.Don) Baker, *L. taliense* Franch., *L. wardii* Stapf ex F.C.Stern, *L. souliei* (Franch.) Sealy. The species of the last "*Lophophorum* group" are regarded as the closest relatives with the genus *Nomocharis*, from which many of them were recently returned back to *Lilium*.

Leucolirion (Trumpet group)

This group is characterized by the funnel shaped, large, white or whitish flowers. The leaves are scattered, and the seeds have immediate epigeal germination. Genetic diversity of a native Taiwanese lily, *L. formosanum,* was earlier studied by RAPD, and significant differences between strains from high and low altitudes were found (Wen and Hsiao 2001). The six species in this group can be divided into two subgroups by their natural occurrence and morphology: *L. regale*, *L. sargentiae* E.H.Wilson, *L. sulphureum* Baker ex Hook.f. and *L. leucanthum* (Baker) Baker originating from Central and Southern China, *L. longiflorum s.l.*, *L. formosanum*, *L. wallichianum s.l.* Schult. & Shult.f. and *L. philippinense* Baker, having even more southern distribution as they originate from Southern Himalayan regions, Northern India and Taiwan (Jefferson-Brown and Howland 1995).

Daurolirion (*L. bulbiferum* and *Dauricum* group)

Lilium bulbiferum is a well-known lily in European gardens. The origin of the cultivars is most likely in central Europe as the wild forms *L. bulbiferum typicum* and its variety *croceum* (Chaix) Pers. grow there in their native habitats. The wild forms of *L. bulbiferum* reproduce via seeds, but the domesticated strains do not produce any seeds and are generally propagated vegetatively (Woodcock and Stearn 1950). The inability for sexual reproduction may be due to strong self-incompatibility that can result from extensive inbreeding. Generally, allelic variation can be lost and inbreeding may increase in small populations. This is due to accumulation of a genetic load through drift (Frankham *et al.* 2002), where specific alleles become more dominating within the small population, and with time, traits can be completely lost or they may become masked by other genes (Wright 1977). In the study by Pelkonen *et al.* (2007), the seedling strains of the *Bulbiferum* group (*L. bulbiferum typicum* and *L. bulbiferum* var. *croceum*) formed two subgroups, as the type form and the variety fell into their own groups. This confirmed the historical classification that was based on morphological differences, classifying the *L. bulbiferum* forms as two varieties of the species (Woodcock and Stearn 1950). The subgroups within *L. bulbiferum s.l.* vary by several characteristics, e.g. the potential to form bulbils (Pelkonen *et al.* 2007).

Some cultivated strains of *L. bulbiferum* morphologically resemble species of a close taxonomic group *Dauricum* (*L. dauricum*, syn. *L. pensylvanicum*, and *L. maculatum*), but phylogenetically they have proved to be more distant (Pelkonen *et al.* 2007). As already suggested by Nishikawa *et al.* (1999), Dubouzet and Shinoda (1999) and Muratovíc *et al.* (2010), it is justified to include the *L. bulbiferum s.l.* into *Daurolirion* or *Sinomartagon*, rather than to *Liriotypus*. The data presented in this paper further suggests that *Daurolirion* does not form a separate group even within *Sinomartagon*, and the existence of this group can therefore be questioned.

CONCLUSION

New methods have brought a multitude of new and more precise information about the relationships of different species and taxonomic groups within the *Lilium* genus. The new methods based on the sequence data derived from either genomic or chloroplast DNA can be considered more accurate than the earlier methods that were based on phenotypic and geographical information, and show in some cases the crossing of geographic boundaries. Still, the conventional approach has proved to be considerably reliable as far as the general organization of the genus is concerned. This may be due to the stability of the species, scarce occurrence of spontaneous interspecific hybridization and the lack of a marked environmental pressure towards the natural habitats of the species. Adaptation to environmental conditions may subject the plant to differentiation through, for example polyploidy (McMillan and Weiler 1959; Ben Fadhel and Boussaid 2004).

Loss of variation, speciation and differentiation can take place in populations of continually small effective sizes (He *et al.* 2000). During the last decades, the main source for variation of *Lilium* species seems to be from human activity. This has become evident along with the tens of thousands of new cultivars and hybrids, which have been produced for ornamental uses. On the other hand, man has also ever increasing impact on the natural populations by destroying their natural habitats and exploiting the natural populations by collecting.

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