

Integrating morphological data and chloroplast genome analyses provides novel insights into the phylogeny of *Lilium* species in China

Zengfu Bai

Northwest Normal University

Jie Fu

Chengdu University of Traditional Chinese Medicine

jiulin Gu

Sichuan College of Traditional Chinese Medicine

Zhihua Zhang

Northwest Normal University

Jiajia Cui

Northwest Normal University

Xiaohui Tang

Sichuan Academy of Grassland Sciences

Tingju Li

Sichuan Academy of Grassland Sciences

Xuelin Chen

chenxuelin63@163.com

Northwest Normal University

Weiji Zhao

Sichuan Academy of Grassland Sciences

Ji Zhang

Northwest Normal University

Research Article

Keywords: Liliaceae, New species, Morphology, Phylogenetic relationships, Taxonomy

Posted Date: August 8th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7136554/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background: The genus *Lilium* comprises plants with combined ornamental, edible, and medicinal values, distributed primarily in the temperate regions of the Northern Hemisphere, subtropical mountainous areas, Southeast Asia, and tropical zones. Although previous studies using various molecular markers have provided significant insights into species delimitation and phylogeography of *Lilium*, the taxonomic status of some species within this genus remains unresolved, necessitating further detailed investigation.

Results: The chloroplast genomes (cpDNAs) of *Lilium* species range in size from 151,655 to 153,235 bp and exhibit a typical quadripartite structure, comprising a large single-copy region (LSC, 81,222–82,496 bp), a small single-copy region (SSC, 17,031–17,792 bp), and two inverted repeat regions (IRs, 26,271–26,990 bp). Twelve highly variable regions were identified: *matK-trnK*, *trnS-trnG*, *rps16*, *trnE-trnT-psbD*, *trnT-trnL*, *trnF-ndhJ*, *trnP-psaJ-rpl33*, *psbE-petL*, *petD-rpoA*, *rpl32-trnL*, *ndhD*, and *rps15-ycf1*. These findings suggest that combining morphological data with complete chloroplast genome sequences is more effective for resolving phylogenetic relationships among *Lilium* species. This study not only advances our understanding of plant diversity within the genus *Lilium* but also provides a critical theoretical foundation and empirical data to support taxonomic research and conservation strategies for these species.

Conclusion: In this study, we analyzed the complete chloroplast genomes of 63 *Lilium* species and proposed a new species, *Lilium jiulongense*, clarified its phylogenetic placement. Additionally, this work provides novel molecular markers and genomic resources to support phylogenetic reconstruction and species delimitation within the genus *Lilium*.

Introduction

The genus *Lilium*, a prominent member of the family Liliaceae, has been of great interest to botanists and horticulturists due to its remarkable diversity and ecological importance. First described by Linnaeus in 1753, this genus comprises approximately 120 species within the Liliaceae family [1–3]. It is distributed across temperate regions of the Northern Hemisphere, subtropical mountain zones, and tropical areas of Southeast Asia, including its mainland regions [4]. Several species, such as *L. amoenum* E.H.Wilson ex Sealy, *L. wardii* Stapf ex Stearn, *L. fargesii* Franch., and *L. regale* E.H.Wilson, are endemic to China. *Lilium* exhibits the highest species diversity in East Asia, particularly within China. These plants are widely cultivated for their significant horticultural, ornamental, edible, and medicinal value [5–6].

The widely accepted classification of the genus *Lilium* by Comber [7] divides *Lilium* into seven sections, namely sect. *Archelirion*, sect. *Daurolirion*, sect. *Leucolirion*, sect. *Liriotypus*, sect. *Martagon*, sect. *Pseudolirium*, and sect. *Sinomartagon*. Recent research has shown that these sectional classification units can result in non-monophyletic plant groups [8–14]. For instance, sect. *Sinomartagon* has been confirmed to be non-monophyletic [14, 15–17]. Additionally, *Nomocharis*, which was once considered a separate genus, is now classified as a subordinate taxon within *Lilium* [18–19]. The discovery of the new

species *Lilium liangiae* Y. M. Yuan et Y. D. Gao serves as an additional bridge connecting the former *Nomocharis* with other members of *Lilium*, enhancing the morphological diversity of the group. Phylogenetic results and morphological clustering both support the classification of *Lilium liangiae* within the Ecristata subclade of *Nomocharis*, with its closest relative being *Lilium gongshanense* [20].

The genomic architecture of chloroplasts is relatively stable [21], yet pronounced interspecific variations in size and sequence exist, providing a valuable tool for phylogenetic studies and the identification of closely allied species [22]. Recently, the rapid advancement of next-generation sequencing has enabled more complete chloroplast genome analyses, which are now frequently used in evolutionary and population genomic studies. Zhou et al. [17] utilized the complete chloroplast genomes of 64 *Lilium* species to reconstruct the phylogenetic scaffold and a time-calibrated phylogenetic framework of the genus. This was done to estimate the biogeographical historical scenarios and evolutionary diversification rates of *Lilium*. The results suggest that ancient climatic changes and geological tectonic activities have jointly shaped the distribution of *Lilium* and driven its evolutionary radiation.

In this study, we sequenced and described in detail a newly identified *Lilium* species, compared it with its close relatives (Table 1), and determined its genomic structure and phylogenetic characteristics. Furthermore, we analyzed the chloroplast genomes of 63 *Lilium* species (Table S1), investigated their molecular characteristics, and performed comparative analyses. Ultimately, we revealed the sequence variations, molecular evolution, and phylogenetic relationships among these 63 *Lilium* species' chloroplast genomes.

Results

Taxonomic treatment

Lilium jiulongense Z.F.Bai, Xue L.Chen & J.L.Gu, *sp. nov.* (Figs. 9).

Type:—CHINA. Sichuan: Jiulong County, Damp stone wall, elev. 2200–2600 m, 30 July 2024, *Zengfu Bai, Xuelin Chen & Jiulin Gu 2024012* (holotype NWTC!; isotypes NWTC!).

Description. Perennial herbs. Bulb ovoid, 1.5–2.1 cm long, 1.6–2.8 cm in diameter; scales white without joint, ovate or lanceolate-ovate. Stem glabrous, 28.5–65 cm tall, 4–5 mm in diameter. Leaves cauline, alternate, linear, 3–15 × 0.3–0.9 cm. Flowers nodding. Tepals margin revolute, dark red, green at the base and dark purple spots. pedicels 2.5–7 cm long, glabrous, base with 1 bracts, bracts linear, 10–40 × 3–4 mm. Tepals 6, in 2 whorls, outer 3 linear or linear-lanceolate, 40–60 × 8–12 mm, inner 3 also linear or linear-lanceolate, 40–60 × 12–15 mm, glabrous; nectaries papillose on both surfaces. Stamens 6, filaments filiform, glabrous, 3.5–4.5 cm long, anthers lunate, dark red-orange, 0.8–1 cm long. Ovary cylindrical, green, 8–14 mm long, 2–3 mm in diameter, style clavate, ca. 4 mm with trilobed stigma, 2 mm long. styles 2–4 cm long.

Phenology:—Flowering July–August, fruiting from September– November.

Etymology:—The Chinese name is proposed as “jiǔ long bǎi hé (九九百何)”.

Distribution and habitat. *Lilium jiulongense* is currently only found in Jiulong county, Sichuan, China. It grows in the cracks of wet stone approximately 2200-2600 m above sea level.

Cp genome organization of *Lilium*

The chloroplast genomes of *Lilium*, which have been sequenced and analyzed, display a typical quadripartite structure consisting of a large single copy (LSC), a small single copy (SSC), and two inverted repeat regions (IRA and IRB) (Fig. 1). The plastome size varies from 151,655 bp in *L. bakerianum* to 153,235 bp in *L. fargesii*, with an LSC region spanning 81,222-82,496 bp and an SSC region ranging from 17,031 to 17,792 bp, separated by two inverted repeat (IR) regions of 26,271–26,990 bp. The three *L. jiulongense* plastomes are approximately 153 kb in size. The overall guanine-cytosine (GC) content is around 37%, with the LSC and SSC regions of all 28 species exhibiting lower GC content than the IR regions. The chloroplast genomes in the 28 samples have a conserved gene content. Most species are annotated as having 129 genes, which include 38 tRNA genes, eight rRNA genes, and 83 protein-coding genes. Twelve species have a total of 83 protein-coding genes, another twelve contain 84 protein-coding genes, and four species possess 85 protein-coding genes (Table S2 and S3).

Structural comparison of *Lilium* cp genomes

The locations of IR/SC junctions were conserved among all cp genomes (Fig. 2). In general, the *rps19* gene extended 23-145 bp into the IRs at the LSC/IRb (JLB) junction. The *ndhF* gene was located in the SSC, 1-55 bp from the SSC/IRb (JSB) border. In *L. fargesii* and *L. xanthellum*, the *ndhF* gene extends into the IRb region by 55 and 1 bp, respectively, crossing the JSB. In *L. jiulongense*, the *ndhF* gene was located at the SSC/IRb (JSB) border. In all cp genomes except *L. pumilum*, the SSC/IRa (JSA) junction was within the *ycf1* gene, with 1222 to 1656 bp of *ycf1* duplicated in the IRb region.

In this study, we identified 674 long repeats in 16 *Lilium* chloroplast genomes, comprising 360 palindromic repeats, 233 forward repeats, 68 reverse repeats, and 13 complement repeats. *L. fargesii* had the most long repeats (64), while *L. tsingtauense*, *L. amabile*, and *L. pumilum* had the fewest (28). There was a significant disparity in the distribution of the four repeat types in *Lilium*; palindromic and forward repeats were more abundant (8–30), whereas complement and reverse repeats were less common (0–13). Among all long repeats, 30-39 bp sequences were most frequent (586), followed by 40-49 bp (130), 50-59 bp (25), and >50 bp (24) (Fig. 3).

In *L. jiulongense*, there were 45 repeats in total, consisting of 17 forward repeats, 5 reverse repeats, and 23 palindromic repeats. We identified a total of 200 to 214 SSRs, among which mononucleotide repeats were the most abundant, ranging from 106 to 115 and accounting for 53 to 53.74% of all SSRs. Trinucleotide repeats came next, ranging from 69 to 76 (34.5% to 35.51%), followed by dinucleotide repeats (10 to 16, 5% to 7.48%), tetranucleotide repeats (7 to 12, 3.5% to 5.61%), pentanucleotide repeats (0 to 5, 0% to 2.34%), and hexanucleotide repeats (0 to 1, 0% to 0.47%). Most mononucleotide SSRs were

A/T repeats, with AAT/ATT repeats being the second most common. Hexanucleotide repeats were only found in the cp genomes of *L. callosum* and *L. fargesii*. These SSRs were predominantly located in the LSC regions. In the cp genome of *L. jiulongense*, we identified 200 SSRs, including 107 mononucleotide repeats (101 A/T repeats and 6 G/C repeats), 6 dinucleotide repeats (all AT repeats), 70 trinucleotide repeats (23 AAT/ATT, 22 AAG/CTT, 8 AAC/GTT, 6 ATC/ATG, 4 AGC/CTG, and 4 AGG/CCT), 11 tetranucleotide repeats (8 AAAT/ATTT, 2 AAAG/CTTT, and 1 AATT/AATT), and 2 pentanucleotide repeats (1 AAAGT/ACTTT and 1 AACTT/AAGTT) (Fig. 4).

Identification of hypervariable regions

This study used the chloroplast genome of *L. jiulongense* as a reference to align and compare the chloroplast genomes of 16 other plant species. The Shuffle-LAGAN model in mVISTA was used to create genetic maps for 16 *Lilium* chloroplast genomes, enabling an examination of their overall gene composition and sequences (Fig. 5). The findings indicate that the 16 *Lilium* species exhibit significant similarities, suggesting a high level of conservation within the chloroplast genomes of these plants. Notably, the IR region is more conserved than the LSC and SSC regions, and coding regions are more conserved than non-coding regions; additionally, intergenic spacers show more variability than coding sequences. To locate sequence divergence hotspots, nucleotide diversity (P_i) values were calculated within 800 bp windows (Fig. 6). The P_i values ranged from 0 to 0.0192. Significant sequence divergence was observed in the following genomic regions: *matK-trnK*, *trnS-trnG*, *rps16*, *trnE-trnT-psbD*, *trnT-trnL*, *trnF-ndhJ*, *trnP-psaJ-rpl33*, *psbE-petL*, *petD-rpoA*, *rpl32-trnL*, *ndhD*, and *rps15-ycf1*. These highly variable genes and intergenic regions may serve as valuable molecular markers for phylogenetic and population genetic studies involving these 16 *Lilium* species.

Codon Usage Analysis

The codon usage and recognition patterns of 16 *Lilium* species were analyzed. The results indicate that 61 different codons encode 20 distinct amino acids, with the other three being stop codons. Codon counts vary from 18068 in *L. amabile* to 22769 in *L. distichum*. Of these, leucine (Leu) is the most abundant amino acid, with a frequency of 10.01–10.40 %, and cysteine (Cys) exhibited the lowest frequency, ranging from 1.12% to 1.16%. (Table. S5). This finding aligns with the outcomes observed in the amino acid coding sequences of chloroplast genomes across the majority of plant species. The analysis of codon-encoded amino acids revealed that leucine (Leu) was the most prevalent, with occurrences ranging from 1810 times in *L. amabile* to 2355 times in *L. speciosum* var. *gloriosoides*. Conversely, cysteine (Cys) was the least frequently encoded amino acid, appearing only 202 times in *L. amabile* and *L. callosum*, and up to 264 times in *L. speciosum* var. *gloriosoides*. These findings are in alignment with the data presented in Figure 7. The Relative Synonymous Codon Usage (RSCU) value of less than 1.00 signifies that the frequency of codon usage is less than expected, while the RSCU value more than 1.00 indicates that the frequency of codon usage is above the expected level[26]. In this research, the RSCU values for 30 codons were found to exceed 1, while the RSCU values for another 32 codons were determined to be less than 1. Furthermore, the RSCU values for methionine and tryptophan

were found to be equal to 1, suggesting that the codons AUG and UGG exhibit no discernible bias or preference. The highest RSCU value was for UUA in leucine, and the lowest was AGC in serine. Among the codons with RSCU value >1, 16 codons ended in U, 13 codons ended in A, and one codon ended in C. These findings suggest a preference for the chloroplast genome codons of *Lilium* species to conclude with A or U (Table S4).

Phylogenetic Analysis

In the phylogenetic analysis of the chloroplast genome (Fig. 8), we used a total of 66 taxa, including 3 outgroup taxa. The results of tree inference using the maximum likelihood (ML) method revealed strong support for the close relationship of the new taxon *L. jiulongense* with *L. duchartrei* and *L. lankongense*. Most of these groups inhabit low-elevation regions. They have similar morphological characteristics: drooping flowers and alternate leaves. Previous studies have indicated that these features represent the most primitive characteristics of the genus *Lilium* [27,28]. In addition, *Lilium* was strongly supported (BS = 100%) as sister to *Notholirion*, *Cardiocrinum*, and *Fritillaria*. In the current study, *Lilium* species were divided into 12 clades based on nrITS and chloroplast genomes, but only a few sections were monophyletic [7,9,11,20,29,30].

Morphological characteristics

Detailed morphological characteristics of five species closely related to *Lilium jiulongense* are shown in Figure 9. The main morphological characteristics of *Lilium jiulongense* are: often forming long stolons; bulb scales white; stems and leaves glabrous and lacking papillae; tepals red with black spots, green at base (Figures B1-B6, Table 1). *Lilium lankongense*: with stolons; bulb scales white; stems and leaves minutely papillate; tepals pink with deep red spots (Figures C1-C6, Table 1). *Lilium duchartrei*: with rhizomes; bulb scales white; stems and leaves slightly papillate; tepals white or pink with reddish purple spots (Figures A1-A6, Table 1).

The remaining species lack stolons:

Lilium nanum: bulb scales white; stems glabrous; leaves papillate; tepals pale purple or purplish red with deep purple spots (Figures D1-D6). *Lilium lophophorum*: bulb scales white; stems glabrous; leaves papillate; flowers yellow, pale yellow, or pale yellowish green, often becoming purplish red after flowering, with very sparse purplish red spots or spotless (Figures E1-E6). *Lilium xanthellum*: bulb scales yellow; stems densely covered with scale-like hairs; leaves papillate; tepals yellow, with or without purple spots (Figures F1-F6).

Table 1. Main differences among *Lilium jiulongense*, *L. lankongense* and *L. duchartrei*.

Characters	<i>Lilium jiulongense</i>	<i>L. lankongense</i>	<i>L. duchartrei</i>
Habitat	Wet cliffs, 2200-2600 m	Alpine grasslands, 1500–3800 m	Forest margins along valleys, grassy slopes, hillsides, 1800–3200 m
Stems	epapillate	papillose	sometimes slightly papillose
Leaves	Linear, 3–15 × 0.3–0.9 cm	oblong, oblong-lanceolate, or lanceolate, 3–10 × 0.5–1.7 cm	lanceolate to oblong-lanceolate, 4.5–5×0.8–1 cm
Flower and Tepals	Nodding, dark red, green at the base and dark purple spots	Nodding, pink, with deep red spots	Nodding, white, with red-purple spots
Nectary	papillose on both surfaces	papillose on both surfaces	papillose on both surfaces

Discussion

Cp genome structural changes in *Lilium*

The complete chloroplast genome of terrestrial plants is more highly conserved, shorter, and more easily obtained than nuclear and mitochondrial genomes (Badenes & Parfitt, 1995). In recent years, it has frequently been used for studies in plant identification, classification, and phylogenetics[31]. In this study, the plastomes of 3 individuals from one species *Lilium* were newly sequenced, annotated and compared. The results indicate that the newly sequenced *Lilium jiulongense*, like other *Lilium* species, possesses a typical tetraploid structure and similar genome size. about 153 kb (151655 bp–153235 bp), and the GC contents of the plastomes were approximately 37%, which were similar to the sequence length and composition of the previously obtained plastomes [15,32]. A comparative analysis of the GC content across various regions of the chloroplast genome in *Lilium* species reveals that the GC content in the inverted repeat region (IR) is higher than that in the large single copy region (LSC) and the small single copy region (SSC). This elevated GC content contributes to the genomic stability, suggesting that the conservation of the IR region may be associated with its higher GC content [33]. By conducting an in-depth analysis of the boundary sequence variations within the large single-copy (LSC), small single-copy (SSC) regions, and the two inverted repeats (IRa and IRb) of the *Lilium* chloroplast genome, we observed that the boundary sequence variations in the *Lilium* chloroplast genome are relatively minor (Figure 2), similar to those observed in *Engelhardia*, indicating a relatively conserved pattern in the boundaries of the inverted repeat regions [34]. In the genus *Lilium*, the distribution of the *ycf1* gene follows a specific pattern akin to that observed in *Engelhardia* and *Aconitum* [24]. The gene is present in two copies within the plastid genome: one copy is located at the SSC/IRa boundary as an intact gene, while the other is found at the IRb/SSC boundary as a pseudogene, *ψycf1*, which no longer encodes a protein. The dual

presence of this gene and the difference in its functional states highlight the complexity of the plastid genome's evolution and the potential influence of genomic structure on gene function and expression.

DNA barcoding is a rapid, accurate, and efficient method for species identification [35]. Liu et al. demonstrated that three new DNA barcodes (ITS + trnP-psaJ-rpl33 + psbB-psbH) can effectively serve as genetic markers for distinguishing species within the genus *Lilium* [36]. In our study, we conducted nucleotide polymorphism analysis on *Lilium* species using complete plastomes. The results indicated that the genetic polymorphism in the IR region was lower than that in the LSC and SSC regions for all species, and coding sequences were more conserved than non-coding sequences, which is similar to findings in most angiosperms [37]. However, we still identified 12 highly variable regions in *Lilium*, including *rps15-ycf1*, *ndhD* and *rps16*.

The codon usage patterns of *Lilium* plastids are vital for exploring its evolution[38]. Synonymous codons, which are multiple codons that encode the same amino acid, do not have equal usage frequencies; this variation is known as codon usage bias[39]. The RSCU metric provides a direct indication of the bias in codon usage [40]. As a result, RSCU values were determined across the entire set of chosen plastomes. Our analysis revealed that a majority of codons conclude with adenine (A) or uracil (U) in their third position (Table S4), aligning with findings from *Zingiber* [23], *Aconitum* [24], *Pulsatilla* [25], and related taxa, which suggests a conserved pattern in the usage of the third nucleotide in plastid genomes of plants [41].

Phylogenetic relationships of *Lilium*

Traditional plant classification relies on morphological characteristics, but these are often influenced by the environment and parallel evolution [42], thus necessitating molecular evidence; in phylogenetic studies, the combination of morphological data is essential for identifying species and inferring their relationships. Therefore, we should integrate chloroplast genome data with morphological traits to analyze the phylogenetic position of the new species *Lilium jiulongense*. Interestingly, in this study, the position of *Lilium jiulongense* is the same in both morphological and cp genome trees. They collectively form the *L. duchartrei* branch [10]. The results indicate that species with similar morphological characteristics tend to cluster together and exhibit higher internal resolution.

Conclusion

In the present investigation, we characterized *L. jiulongense* through sequencing and description, shedding light on the morphological diversity within the *L. duchartrei* clade. Furthermore, by conducting a comparative analysis of 16 *Lilium* chloroplast genomes, we pinpointed 12 regions exhibiting high variability, which may be utilized as potential molecular markers for species discrimination. The combined analysis of morphological characteristics and phylogeny strongly supports the inferred interspecific relationships. Consistent with other taxonomic groups, the flowering stage emerged as the optimal period for the precise identification of *Lilium* species. Our study contributes to the genomic

repository of *Lilium*, facilitating subsequent in-depth phylogenetic analyses and elucidating the intricate relationships within the genus.

Material and methods

Plant materials and DNA extraction

In this study, we sequenced the plastid genomes of three samples from one new species, with an additional 62 plastid genomes obtained from GenBank (<https://www.ncbi.nlm.nih.gov>). A total of 66 individuals were included (Table S1). Genomic DNA was isolated from silica-dried leaf tissues, with voucher specimens deposited in the herbarium of the College of Life Sciences, Northwest Normal University (NWTC). The newly sequenced species samples were collected from Sichuan Province, China. BZF and CXL officially identified the samples. No specific permits were required for the related localities/activities, as they complied with local regulatory requirements. Detailed voucher information and collection locality details for the newly sequenced species are provided in Table 1. Genomic total DNA was extracted using a modified CTAB method[43]. The genomic total DNA was used for library preparation and paired-end sequencing (PE) on the Novogene company's Novaseq instrument in Guangzhou, China. The raw data amounted to approximately 5Gb. Furthermore, *L. jiulongense* was collected at high, medium, and low altitudes, designated as *Lilium jiulongense* SHB, *Lilium jiulongense* ZHB, and *Lilium jiulongense* XHB, respectively (Figure 1).

Cp genomes assembly and annotation

Chloroplast splicing was performed using the software GetOrganelle [44]. Use the online annotation software Geseq (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>) [45] and CPGAVAS2 (<http://47.96.249.172:16019/analyzer/annotate>) [46] to determine the starting positions and IR regions of the assembled chloroplast genomes, and annotate the genes on them. Finally, manual proofreading is required to confirm whether there are any errors in the annotations.

Codon usage and RNA editing sites

For the generation of a hierarchical clustering heatmap depicting RSCU values across the 18 *Lilium* chloroplast genomes, we leveraged the R statistical environment, version 4.3.0 (<https://www.R-project.org>) as illustrated in Figure 2b. To prognosticate potential RNA editing sites within these chloroplast genomes, we engaged the Predictive RNA Editor for Plants (PREP) (<http://prep.unl.edu/>) online toolkit, which facilitates the identification of putative RNA editing sites in protein-coding genes, employing a significance threshold of 0.8.

Phylogenetic analyses

This study conducted phylogenetic analysis based on chloroplast genome data from 28 *Lilium* species. Sequence alignment was performed using MAFFT v7, followed by filtering of ambiguous sites in the chloroplast genome alignment with Gblocks v0.9b [47] under default parameters. Modeltest v3.7

[48] identified the optimal nucleotide substitution model as GTR+I+G for the complete plastid dataset. Maximum likelihood (ML) analysis was implemented in IQ-TREE v1.6.12 with 1000 bootstrap replicates under default settings, incorporating one cold chain and three heated chains, sampled every 1000 generations across two parallel runs. After discarding the initial 25% of samples as burn-in, a 50% majority-rule consensus tree was constructed from the remaining samples. Ultimately, a robust phylogenetic framework for *Lilium* was established with strong statistical support.

Morphological observations

To determine the morphological position of the new species *Lilium jiulongense* within the genus *Lilium*, we consulted Flora of China [4, 30], Watanabe et al. [49], and online databases (high-resolution digitized images from the Chinese Virtual Herbarium and the Plant Photo Bank of China). Taking into account key taxonomic characteristics within the genus *Lilium*, we selected five closely related species of *L. jiulongense* for comparison. Morphological features including bulbs, flowers, and leaves were observed. We then photographed both the overall architecture and detailed morphological features of individual parts using digital cameras to provide comprehensive documentation.

Declarations

Acknowledgements

We thank the Herbarium of the NWTC for specimen preservation, all members of this study for their efforts in field investigations, and Jiulin Gu for providing photographs.

Authors' contributions

BZF: performed data analysis and drafted the manuscript; FJ: conceived the study, designed the experiments, reviewed the paper; GJL: collected the species of sample, performed the experiments; ZZH: conceived the study, designed the experiments; ZWJ and CXL: conceived the study, funding acquisition, collected and identified the species of sample, data curation. ZJ and CJJ: conceived the study, funding acquisition, reviewed the manuscript critically. All authors have read and agreed with the contents of the manuscript.

Funding

Common Key Technologies for the Enhancement of Lanzhou Lily Industry: Research, Development, and Integrated Application (GXH20220617-5); Core Generic Technologies for Upgrading the Lanzhou Lily Industry: R&D and Systemic Implementation (Lankezi 2023.69); Germplasm Innovation and Industrial Upgrading of Lanzhou Lily: R&D and Application of Core Generic Technologies (2023CYZC-13); 2023 Central Government Fund for Forestry and Grassland Ecological Protection and Restoration—Sichuan Forestry and Grassland Technology Innovation Team Program (CXTD2024005); the Gansu Province Key Research and Development Program Project: Technical Development and Application of Microbial-Essential Oil Composite Formulation for Controlling Fusarium Wilt in Lanzhou Lily 23YFNA0016

and the Lanzhou City Youth Science;Technology Talent Innovation Project: Study on the Regulatory Mechanisms of Typical Endophytic Degrading Bacteria on the Growth and Quality of Lanzhou Lily under Continuous Cropping Conditions (2023-QN-176).

Availability of data and materials

The complete chloroplast genomes generated during the current study were deposited in NCBI database (Accession number: PV916038)

Ethics approval and consent to participate

The authors declare that the collection of plant materials for this study complies with relevant institutional, national and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Linnaeus, C. (1753). *Species plantarum* (Stockholm: Salvius), 302.
2. POWO (2025) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew Published on the Internet. Available online at: <http://www.plantsoftheworldonline.org/>.
3. Wang T, Yuan Y, Zhou T-H, Gao Y (2025) *Lilium huanglongense* (Liliaceae): a newly-discovered species in north-western Sichuan, China. *PhytoKeys* 252: 9–24.
4. Liang, S.Y. and M.N. Tamura. 2000. *Lilium* Linnaeus. – In: Wu, Z. Y. and Raven P. H. (eds), *Flora of China*. Vol. 24: 135–149. Science Press; Miss. Bot. Gard. Press, Missouri.
5. Woodcock, H.B.D. & Stearn, W.T. (1950) *Lilies of the world: their cultivation and classification*. Country Life, London, 431 pp.
6. Rong, L.P. & Lei, J.J. & Wang, C. (2011) Collection and evaluation of the genus *Lilium* resources in Northeast China. *Genetic Resources and Crop Evolution* 58: 115–123. <https://doi.org/10.1007/s10722-010-9584-2>.
7. Comber, H.F. (1949) A new classification of the genus *Lilium*. in: Chittenden, F.J. (Ed.) *Royal Horticultural Society Lily Year Book*. Roal Horticultural Society, London, pp. 85–105.
8. Nishikawa, T., Okazaki, K., Uchino, T., Arakawa, K., and Nagamine, T. (1999). A molecular phylogeny of *Lilium* in the internal transcribed spacer region of nuclear ribosomal DNA. *J. Mol. Evol.* 49, 238–249. doi: 10.1007/PL00006546

9. Gao, Y.-D., Harris, A., Zhou, S.-D., and He, X.-J. (2013). Evolutionary events in *Lilium* (including *Nomocharis*, Liliaceae) are temporally correlated with orogenies of the Q–T plateau and the Hengduan Mountains. *Mol. Phylogenet. Evol.* 68, 443–460. doi: 10.1016/j.ympev.2013.04.026
10. Du, Y.-p., He, H.-b., Wang, Z.-x., Li, S., Wei, C., Yuan, X.-n., et al. (2014). Molecular phylogeny and genetic variation in the genus *Lilium* native to China based on the internal transcribed spacer sequences of nuclear ribosomal DNA. *J. Plant Res.* 127, 249–263. doi: 10.1007/s10265-013-0600-4.
11. Gong, X., Hung, K.H., Ting, Y.W., Hsu, T.W., Malikova, L., Tran, H.T., Huang, C.L., Liu, S.H. & Chiang, T.Z. (2017) Frequent gene flow blurred taxonomic boundaries of sections in *Lilium* L. (Liliaceae). *PLoS One* 12: e0183209. <https://doi.org/10.1371/journal.pone.0183209>
12. Huang, J., Yang, L.-Q., Yu, Y., Liu, Y.-M., Xie, D.-F., Li, J., et al. (2018). Molecular phylogenetics and historical biogeography of the tribe Lilieae (Liliaceae): bi-directional dispersal between biodiversity hotspots in Eurasia. *Ann. Bot.* 122, 1245–1262. doi: 10.1093/aob/mcy138
13. Kim, J. S., and Kim, J. H. (2018). Updated molecular phylogenetic analysis, dating and biogeographical history of the lily family (Liliaceae: Liliales). *Bot. J. Linn. Soc.* 187, 579–593. doi: 10.1093/botlinnean/boy031
14. Li, J., Cai, J., Qin, H.-H., Price, M., Zhang, Z., Yu, Y., et al. (2022a). Phylogeny, age, and evolution of tribe Lilieae (Liliaceae) based on whole plastid genomes. *Front. Plant Sci.* 12, 699226. doi: 10.3389/fpls.2021.699226
15. Du, Y.P., Bi, Y., Yang, F.P., Zhang, M.F., Chen, X.Q., Xue, J. & Zhang, X.H. (2017) Complete chloroplast genome sequences of *Lilium*: insights into evolutionary dynamics and phylogenetic analyses. *Scientific reports* 7: 5751. <http://doi.org/10.1038/s41598-017-06210-2>
16. Kim, H.T., Lim, K.B. & Kim, J.S. (2019) New insights on *Lilium* phylogeny based on a comparative phylogenomic study using complete plastome sequences. *Plants* 8: 547. <https://doi.org/10.3390/plants8120547>
17. Zhou, N., Miao, K., Liu, C., Jia, L., Hu, J., Huang, Y. & Ji, Y. (2023) Historical biogeography and evolutionary diversification of *Lilium* (Liliaceae): new insights from plastome phylogenomics. *Plant Diversity*. <https://doi.org/10.1016/j.pld.2023.07.009>
18. Gao, Y.D., Hohenegger, M., Harris, A., Zhou, S.D., He, X.J. & Wan, J. (2012) A new species in the genus *Nomocharis* Franchet (Liliaceae): evidence that brings the genus *Nomocharis* into *Lilium*. *Plant Systematics and Evolution* 298: 69–85. <https://doi.org/10.1007/s00606-011-0524-1>
19. Gao, Y. D., and Gao, X. F. (2016). Accommodating *Nomocharis* in *Lilium* (Liliaceae). *Phytotaxa* 277, 205–210. doi: 10.11646/phytotaxa.277.2.8
20. Yuan Y, Gao Y. 2024. *Lilium liangiae*, a new species in the genus *Lilium* (Liliaceae) that reveals parallel evolution within morphology. *Front Plant Sci.* 15:1371237. doi:10.3389/fpls.2024.1371237.
21. Sugiura, M. (1992). The Chloroplast Genome. *Plant Mol. Biol.* 19, 149–168. doi:10.1007/bf00015612
22. Chen, X.L., Zhou, J.G., Cui, Y.X., Wang, Y., Duan, B.Z. and Yao, H. (2018) Identification of *Ligularia* Herbs Using the Complete Chloroplast Genome as a Super-Barcode. *Frontiers in Pharmacology*, 9, Article No. 695. <https://doi.org/10.3389/fphar.2018.00695>

23. Jiang, D., Cai, X., Gong, M. et al. Complete chloroplast genomes provide insights into evolution and phylogeny of *Zingiber* (Zingiberaceae). BMC Genomics 24, 30 (2023).
<https://doi.org/10.1186/s12864-023-09115-9>
24. Xue, H., Xing, Y., Bian, C. et al. Comparative analysis of chloroplast genomes of *Pulsatilla* species reveals evolutionary and taxonomic status of newly discovered endangered species *Pulsatilla saxatilis*. BMC Plant Biol 24, 293 (2024). <https://doi.org/10.1186/s12870-024-04940-w>
25. Xia C, Wang M, Guan Y and Li J (2022) Comparative Analysis of the Chloroplast Genome for *Aconitum* Species: Genome Structure and Phylogenetic Relationships. Front. Genet. 13:878182. doi: 10.3389/fgene.2022.878182
26. Sharp, P. M., Tuohy, T. M. F., and Mosurski, K. R. (1986). Codon Usage in Yeast: Cluster Analysis Clearly Differentiates Highly and Lowly Expressed Genes. Nucl. Acids Res. 14, 5125–5143. doi:10.1093/nar/14.13.5125
27. Gao, Y. D., Harris, A. J., and He, X. J. (2015). Morphological and ecological divergence of *Lilium* and *Nomocharis* within the Hengduan Mountains and Qinghai-Tibetan Plateau may result from habitat specialization and hybridization. BMC Evol. Biol. 15, 147. doi: 10.1186/s12862-015-0405-2
28. Liu, C. Q., Gao, Y. D., Niu, Y., Xiong, Y. Z., and Sun, H. (2019). Floral adaptations of two lilies: implications for the evolution and pollination ecology of huge trumpet-shaped flowers. Am. J. Bot. 106, 622–632. doi: 10.1002/ajb2.1275
29. Tuyl, J. M. V., Arens, P., Shahin, A., Marasek-Ciołakowska, A., Barba-Gonzalez, R., Kim, H. T., et al. (2018). “*Lilium*,” in Ornamental Crops, Handbook of Plant Breeding, ed. V. H. Johan (Cham: Springer International Publishing AG), 481–512.
30. Liang, S. Y. (1980). “Anagiospermae, Monocotyledoneae Liliaceae (I),” in Flora Reipublicae Popularis Sinicae (Science Press, Beijing), 116–157.
31. Cao, DL., Zhang, XJ., Xie, SQ. et al. Application of chloroplast genome in the identification of Traditional Chinese Medicine *Viola philippica*. BMC Genomics 23, 540 (2022).
<https://doi.org/10.1186/s12864-022-08727-x>
32. Liu HY, Yu Y, Deng YQ, et al. The Chloroplast Genome of *Lilium henrici*: Genome Structure and Comparative Analysis. Molecules. 2018; 23(6):1276.
33. Kaila T, Chaduvla PK, Saxena S, et al. Chloroplast genome sequence of Pigeonpea (*Cajanus cajan* (L.) Millspaugh) and *Cajanus scarabaeoides* (L.) thouars: genome organization and comparison with other Legumes. Front Plant Sci. 2016;9(7):1847.
34. Huang Y, Jin XJ, Zhang CY, Li P, Meng HH, Zhang YH. Plastome evolution of *Engelhardia* facilitates phylogeny of Juglandaceae. BMC Plant Biol. 2024 Jul 6;24(1):634.
35. Kress WJ. Plant DNA barcodes: applications today and in the future. J Syst Evol. 2017;55(4):291–307.
36. Liu Y, Zhang M, Chen X, et al. Developing an efficient DNA barcoding system to differentiate between *Lilium* species. BMC Plant Biol. 2021 Oct 13;21(1):465.

37. Chi XF, Zhang FQ, Dong Q, et al. Insights into comparative genomics, codon usage bias, and phylogenetic relationship of species from Biebersteiniaceae and Nitrariaceae based on complete chloroplast genomes. *Plants (Basel)*. 2020;9(11):1605. <https://doi.org/10.3390/plants9111605>.
38. Li G, Zhang L, Xue P. Codon usage pattern and genetic diversity in chloroplast genomes of *Panicum* species. *Gene*. 2021;802:145866. <https://doi.org/10.1016/j.gene.2021.145866>
39. Rao YS, Wu GZ, Wang ZF, et al. Mutation bias is the driving force of codon usage in the *Gallus gallus* genome. *DNA Res*. 2011;18(6):499–512.
40. LaBella AL, Opulente DA, Steenwyk JL, et al. Variation and selection on codon usage bias across an entire subphylum. *PLOS Genet*. 2019;15(7):1008304. <https://doi.org/10.1371/journal.pgen.1008304>.
41. Hao BQ, Xia YY, Ye H, et al. Analysis on codon usage bias of the chloroplast genome of *Camellia osmanth*. *Journal of Central South University of Forestry & Technology*. 2022;42:178–86.
42. Wu LW, Nie LP, Xu ZC, et al. Comparative and phylogenetic analysis of the complete chloroplast genomes of three *Paeonia* Section *Moutan* species (Paeoniaceae). *Front Genet*. 2020;11:980. <https://doi.org/10.3389/fgene.2020.00980>.
43. Allen GC, Flores Vergara MA, Krasynanski S, Kumar S, Thompson WF. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nat Protoc*. 2006;1:2320–5.
44. Jian-Jun Jin*, Wen-Bin Yu*, Jun-Bo Yang, Yu Song, Ting-Shuang Yi, De-Zhu Li. 2018. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *bioRxiv*, 256479.
45. Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R and Greiner S (2017) GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Research* 45: W6-W11.
46. Liu, C., Shi, L., Zhu, Y. Chen, H., Zhang, J., Lin, X., Guan, X. CpGAVAS, an integrated web server for the annotation, visualization, analysis, and GenBank submission of completely sequenced chloroplast genome sequences. *BMC Genomics* 13, 715 (2012). <https://doi.org/10.1186/1471-2164-13-715>
47. Talavera, G., and Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biol.* 56, 564–577. doi: 10.1080/10635150701472164
48. Posada D. ModelTest Server: a web-based tool for the statistical selection of models of nucleotide substitution online. *Nucleic Acids Res*. 2006;34:700–03. <https://doi.org/10.1093/nar/gkl042>.
49. Watanabe, S. T., Hayashi, K., Arakawa, K., Fuse, S., Nagamasu, H., Ikeda, H., et al. (2021). Biosystematic studies on *Lilium* (Liliaceae) I. Phylogenetic analysis based on chloroplast and nuclear DNA sequences and a revised infrageneric classification. *Acta Phytotaxonomica Geobotanica* 72, 179–204. doi: 10.18942/apg.202107

Table

Table 1 List of 3 species of *Lilium* sequenced in this study

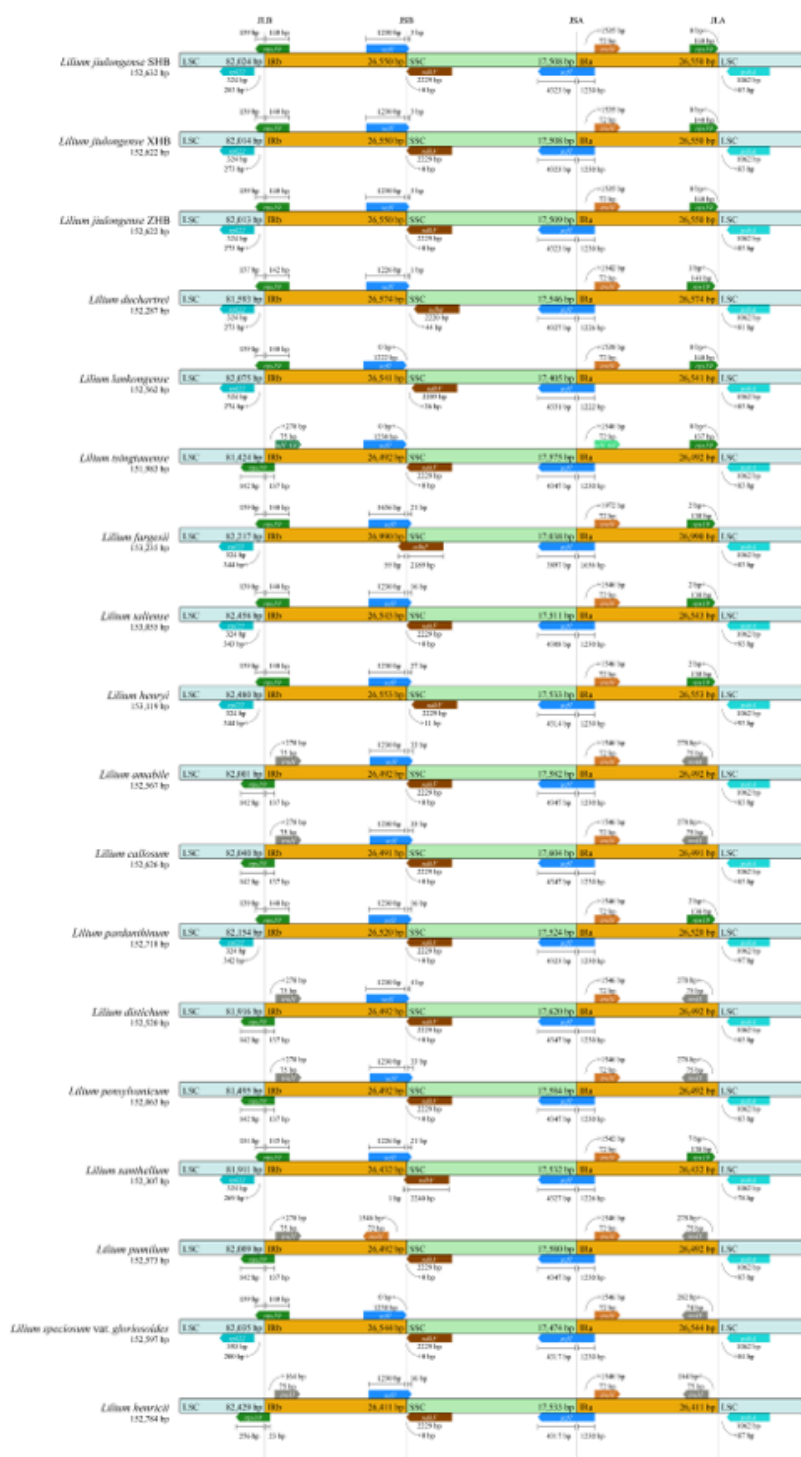


Figure 2

Comparisons of LSC, SSC and IR regions boundaries among 16 chloroplast genomes.

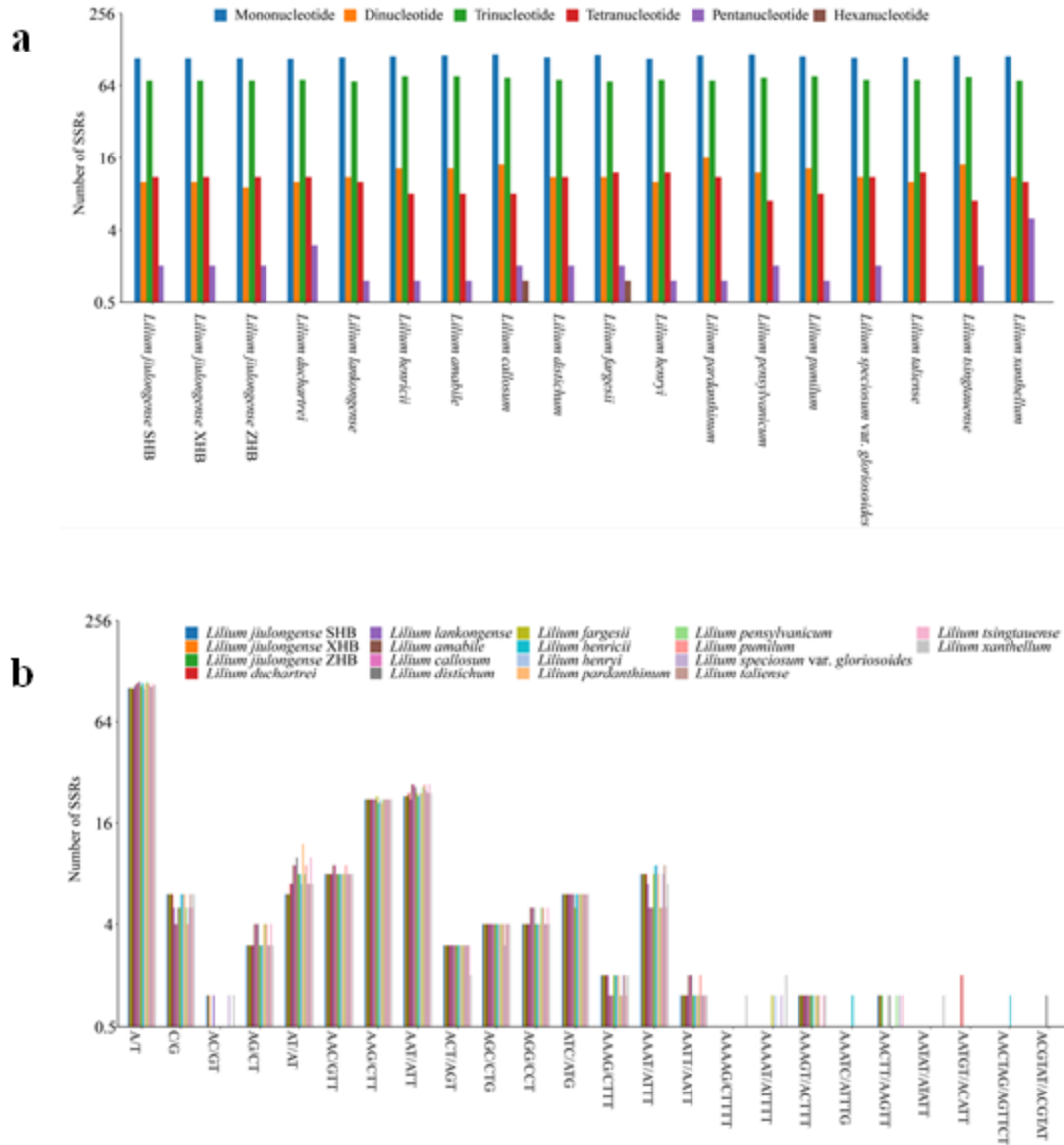


Figure 4

Comparison of the simple sequence repeats (SSRs) among sixteen *Lilium* species. **a** the number of different SSR types. **b** the frequency of the identified SSRs in different repeat class types.



Figure 5

Comparative plots of percent sequence identity of 16 chloroplast genomes in *Lilium*.

Codon content of all protein coding genes. **a** amino acids and stop codons proportion in protein coding sequences of 16 sequenced chloroplast genomes and **b** heat map analysis for codon distribution of all protein coding genes of 16 sequenced chloroplast genomes. Red colour indicates higher RSCU values and blue colour indicates lower RSCU values

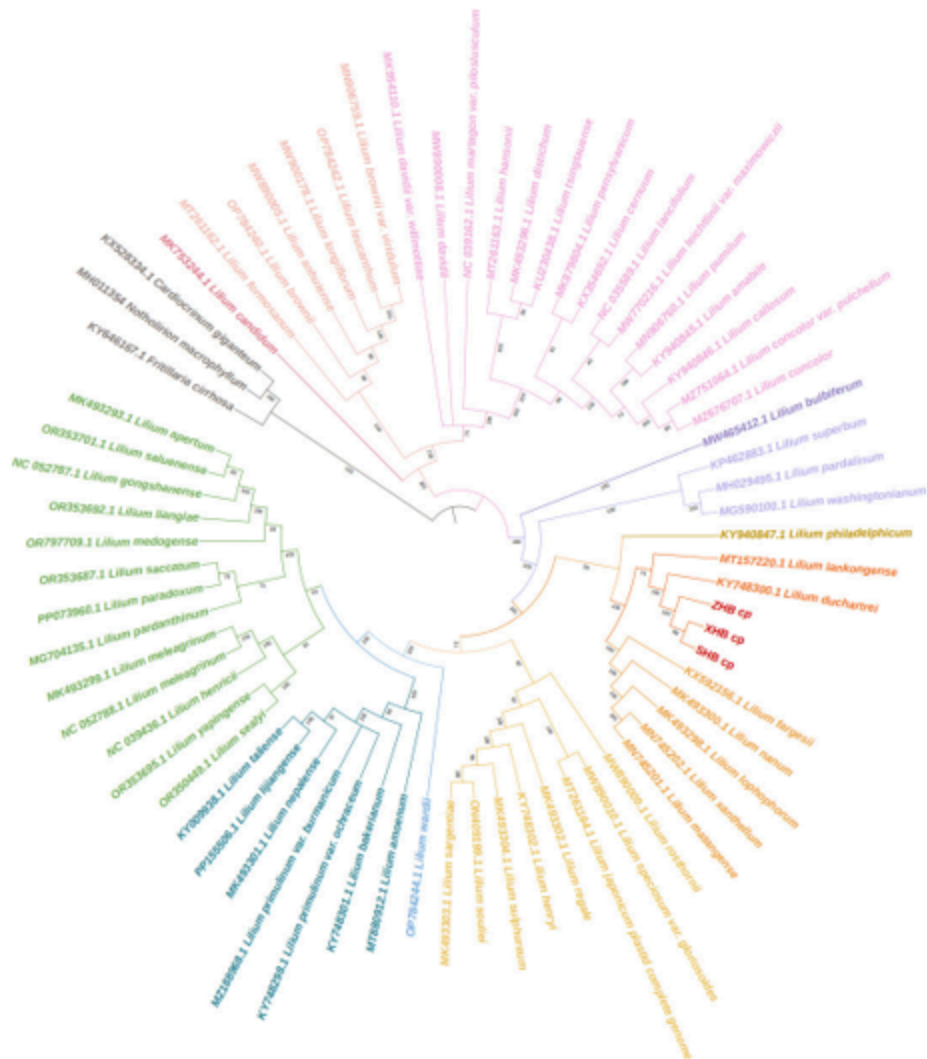


Figure 8

Phylogenetic trees constructed by maximum likelihood based on 63 chloroplast genomes within the genus *Lilium*. The new species, *Lilium jiulongense* is marked in red.

Image not available with this version

Figure 9

Detailed Morphological Characteristics of Six *Lilium* Species by Plant Part. 1. Habit. 2. Bulb. 3. Leaves. 4. Inflorescence. 5. Flower. 6. Inner Tepals.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)