

A complete chloroplast genome of *Sedum lushanense* S. S. Lai 2004 (Crassulaceae: Crassuloideae)

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ABSTRACT

We determined the complete chloroplast genome sequence of *Sedum lushanense* S. S. Lai 2004. The genome was 148,691 bp in length, including a large single copy (LSC; 80,497 bp), a small single copy (SSC; 16,620 bp), and two inverted repeats (IR; 25,787 bp) regions. It contained 84 coding gene sequences (CDS), 34 transfer RNA (tRNA) genes, and eight ribosomal RNA (rRNA) genes. A maximum likelihood phylogenetic analysis revealed a close relationship between *S. lushanense* and *S. lineare*. Therefore, our study provided new genetic information on *S. lushanense*, contributing to a better understanding of its relationship with other related species and the evolutionary history of Crassulaceae.

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Introduction

The family Crassulaceae is divided into three subfamilies, namely Crassuloideae, Kalanchoideae, and Sempervivoideae (Thiede and Eggli 2007). It comprises of approximately 1400 species and 34 genera worldwide (Carrillo-Reyes et al. 2009). Species within this family have adapted to limited water environments through the photosynthetic pathway of crassulacean acid metabolism (CAM) (Liu et al. 2023). *Sedum* Linnaeus is the largest genus in the family Crassulaceae and encompasses approximately 470 species (Fu and Ohba 2001). The genus thrives in temperate and subtropical environments, with primary centers of diversity in the Mediterranean Sea, Central America, the Himalayas, and East Asia (Stephenson 1994; Thiede and Eggli 2007). In China, 121 *Sedum* species have been documented, of which 91 are recognized as endemic (Fu and Ohba 2001). Notably, *Sedum lushanense* S. S. Lai stands out as a species exclusive to China, confined to its designated type locality, Mount Lushan (Liang et al. 2014). This endemism highlights the unique ecological niche and geographic restrictions of this particular *Sedum* species. Recently, *parallel situ* conservation was proposed to integrate the elements of both *in-situ* and *ex-situ* conservation (Feng et al. 2023). Endemic species, such as *S. lushanense*, are ideal for studying the efficiency of *parallel situ* conservation in plants (Feng et al. 2023). However, the scarcity of genetic resources has limited conservation research on this species. In the present study, we aimed to

characterize the complete chloroplast genome of *S. lushanense* and infer its phylogenetic position in the genus *Sedum*.

Material and methods


Plant material

Sedum lushanense was collected from its type locality, Mount Lushan, Jiangxi Province, China (29°33'10.17" N 115°57'42.70" E). A voucher specimen was deposited in the Herbarium of the Lushan Botanical Garden, Chinese Academy of Sciences (LBG) (<http://lbgl.sbg.cn/>, PENG Yansong, pengys@lsbg.cn) under voucher number LTJ2023008.

DNA extraction and chloroplast genome assembly

Total genomic DNA was extracted from fresh leaves using a modified CTAB-chloroform protocol (Doyle and Doyle 1987). We constructed libraries with fragmented DNA, 500 bp in size, and subsequently sequenced them on an Illumina HiSeq X Ten platform using the PE-150 bp protocol. Raw genomic data were deposited in the NCBI Sequence Read Archive under the accession number PRJNA1064019. FASTX-Toolkit v0.0.14 was used to remove adapter sequences from the raw reads and filter out low quality sequences (Q20 < 90%). Clean reads were used for *de-novo* assembly of the chloroplast genome using NOVOplasty v4.3.1 (Dierckxsens et al. 2017). Bowtie2 and Samtools were used to align the assembly and

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verify the coverage, and Matplotlib for Python was employed to visualize the coverage map (Ni et al. 2023). Genome annotation was performed using a combination of GeSeq (Tillich et al. 2017) and CPGAVAS2 (Shi et al. 2019). The complete chloroplast genome was deposited in GenBank (accession no. PP109813).

Phylogenetic analysis

For phylogenetic analysis, we selected 25 Crassulaceae species, including 10 *Sedum* species and one outgroup species (*Penthorum chinense*), to construct a phylogenetic tree. All sequence data, excluding those of *S. lushanense*, were obtained from GenBank. Sequences of the complete chloroplast genomes were aligned using MAFFT v7.313 (Katoh et al. 2002). A maximum likelihood (ML) tree was constructed using the IQ-TREE program (Nguyen et al. 2015) based on the GTR+F+R3 model, with 1000 bootstrap replicates. The phylogenetic tree was visualized using the iTOL tool (Letunic & Bork 2021).

Results

The habitat and basic characteristics of *S. lushanense* are shown in Figure 1A, B, and C. The complete chloroplast genome of *S. lushanense* was 148,691 bp in length, with a GC content of 37.92% (Figure 2). Of the 6,339,590 reads obtained, 251,763 (4.0%) contributed to the chloroplast genome assembly. The mean coverage was $1022.64\times$ (Figure S3). The chloroplast genome exhibited a typical quadripartite structure, including a large single copy (LSC; 80,497 bp), a small single copy (SSC; 16,620 bp), and two inverted repeats (IR; 25,787 bp) regions. A total of 126 genes were identified, comprising of 84 protein-coding genes, 34 tRNA genes, and eight rRNA genes (Table 1). The IR regions encompassed all the duplicated genes, including six protein-coding genes (*ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12*, and *ycf2*), seven tRNA genes (*trnA-UGC*, *trnI-CAU*, *trnI-GAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*), and four rRNA genes (*rrn4.5S*, *rrn5S*, *rrn16S*, and *rrn23S*). Notably, the *ycf1* gene was situated at the

junction of the IR and SSC regions (Figure 2). The chloroplast genome of *S. lushanense* harbored 20 genes with introns, 16 containing one intron each, and *rps12*, *clpP*, and *ycf3* containing two introns (Figure S1, Tables S1 and S2). The trans-spliced nature of the *rps12* gene, located in the IR (IRa and IRb) and LSC regions, was confirmed (Figure S2).

The phylogenetic tree based on protein-coding gene sequences provided robust support for studying the relationships within the Crassulaceae family, exhibiting high bootstrap values ranging from 88% to 100% (Figure 3). The *Crassula* clade was identified as the basal group of the Crassulaceae family. The *Kalanchoe* clade was sister to *Adromischus*. The *Telephium* clade comprised of two subclades, with *Rhodiola* and *Phedimus* forming one subclade, and *Orostachys* and *Hylotelephium* forming the other. *Rosularia*, *Aeonium*, and *Sempervivum* formed a monophyletic clade each with high bootstrap values (100%). These three clades were sisters to the *Acre* clade. The phylogeny of Crassulaceae identified in this study was consistent with that reported previously (Kim & Kim 2020; Liu et al. 2023).

Discussion and conclusion

In this study, we reported the complete chloroplast genome of *S. lushanense*, an endemic species exclusive to Mount Lushan. Chloroplast genome sequencing revealed that the IR regions included all duplicated genes, which is common for chloroplast genomes across various eukaryotic species (Lavi et al. 2018). Furthermore, the structure and gene content of the *S. lushanense* chloroplast genome closely resembled those of other members in the Crassulaceae family (Chang et al. 2020; Chen et al. 2022; Liu et al. 2023). Our phylogenetic analysis based on protein-coding gene sequences revealed the phylogenetic position of *S. lushanense* within the family Crassulaceae. Consistent with previous studies, our results supported the classification system of seven clades within the family Crassulaceae (Liu et al. 2023).

The family Crassulaceae most likely originated in South Africa and Madagascar, with species in the *Acre* clade (primarily *Sedum* species) dispersed from the Mediterranean



Figure 1. Photographs of *Sedum lushanense*. A, whole plant; B, leaves; C, flowers. Wildlife photos were taken by En-dian Yang in the Chinese city of Jiujiang, Jiangxi Province (29°33'10.17" N 115°57'42.70" E).

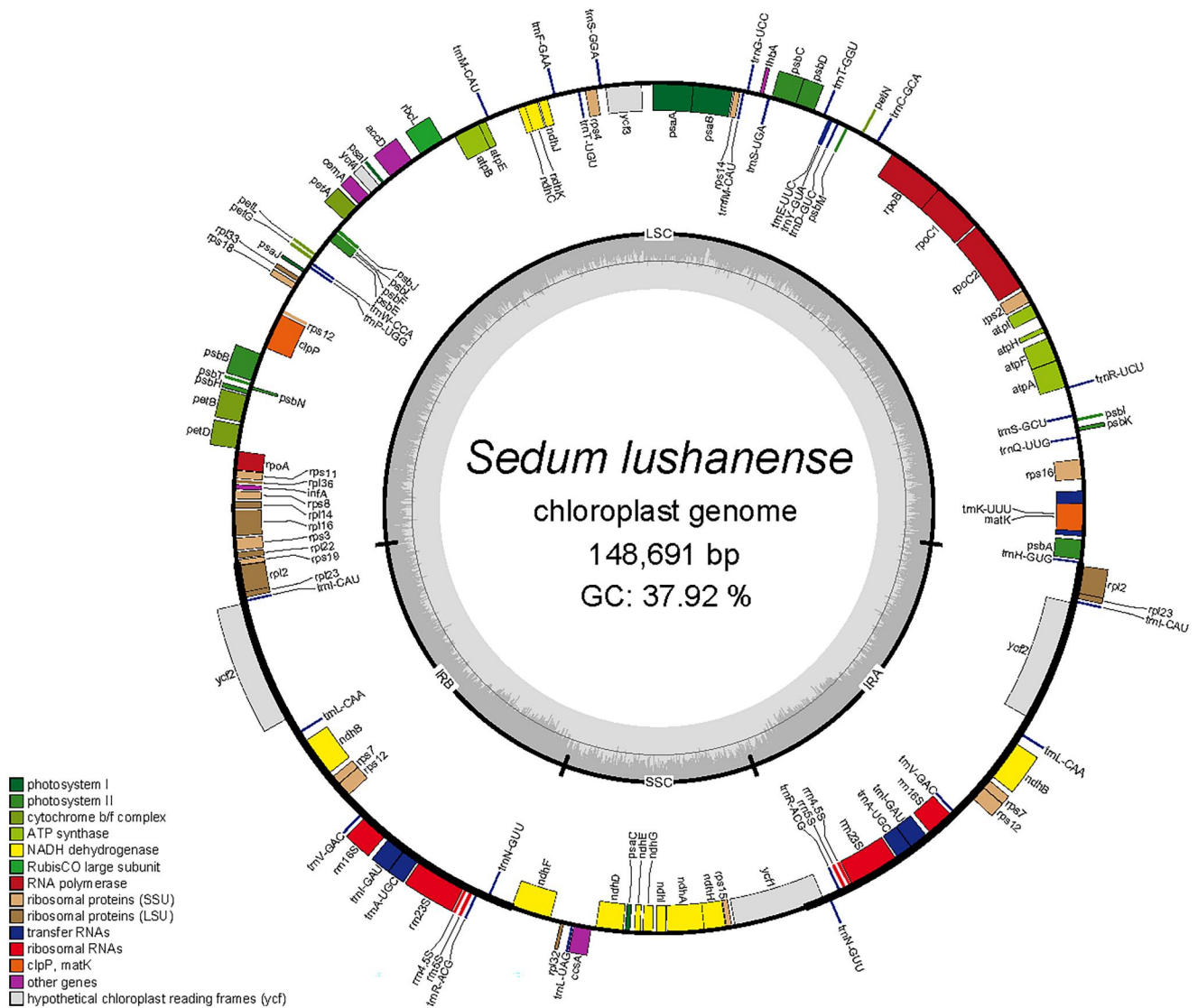


Figure 2. Map of the chloroplast genome of *Sedum lushanense*. Genes with different functional groups are colored and designated. The genes inside and outside of the outer circle are transcribed in clockwise and counterclockwise directions, respectively. The line chart in gray shows the GC content along the genome. LSC: large single copy region; SSC: small single copy region; IRA and IRB: inverted repeat regions.

Table 1. The list of genes in the chloroplast genome of *Sedum lushanense*.

Function (number)	Gene names
CDS (84)	
Subunits of photosystem I (5)	<i>psaA, psaB, psaC, psal, psaJ</i>
Subunits of photosystem II (14)	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT</i>
Subunits of NADH dehydrogenase (12)	<i>ndhA*, ndhB* (×2), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
Subunits of cytochrome b/f complex (6)	<i>petA, petB*, petD*, petG, petL, petN</i>
Subunits of ATP synthase (6)	<i>atpA, atpB, atpE, atpF*, atpH, atpI</i>
Large subunit of rubisco (1)	<i>rbcl</i>
Proteins of large ribosomal subunit (10)	<i>rpl14, rpl16*, rpl2* (×2), rpl22, rpl23 (×2), rpl32, rpl33, rpl36</i>
Proteins of small ribosomal subunit (14)	<i>rps11, rps12** (×2), rps14, rps15, rps16*, rps18, rps19, rps2, rps3, rps4, rps7 (×2), rps8</i>
Subunits of RNA polymerase (4)	<i>rpoA, rpoB, rpoC1*, rpoC2</i>
Other genes (6)	<i>matK, clpP**, cema, accD, ccsA, infA</i>
Proteins of unknown function (6)	<i>hcbA, ycf1, ycf2 (×2), ycf3**, ycf4</i>
Transfer RNAs (34)	<i>trnA-UGC* (×2), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-UCC, trnH-GUG, trnI-CAU (×2), trnI-GAU* (×2), trnK-UUU*, trnL-CAA (×2), trnL-UAG, trnM-CAU, trnN-GUU (×2), trnP-UGG, trnQ-UUG, trnR-ACG (×2), trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC (×2), trnW-CCA, trnY-GUA, trnM-CAU</i>
Ribosomal RNAs (8)	<i>rrn16S (×2), rrn23S (×2), rrn4.5S (×2), rrn5S (×2)</i>

Gene*: gene with one intron; Gene**: gene with two introns; Gene (×2): duplicated gene.

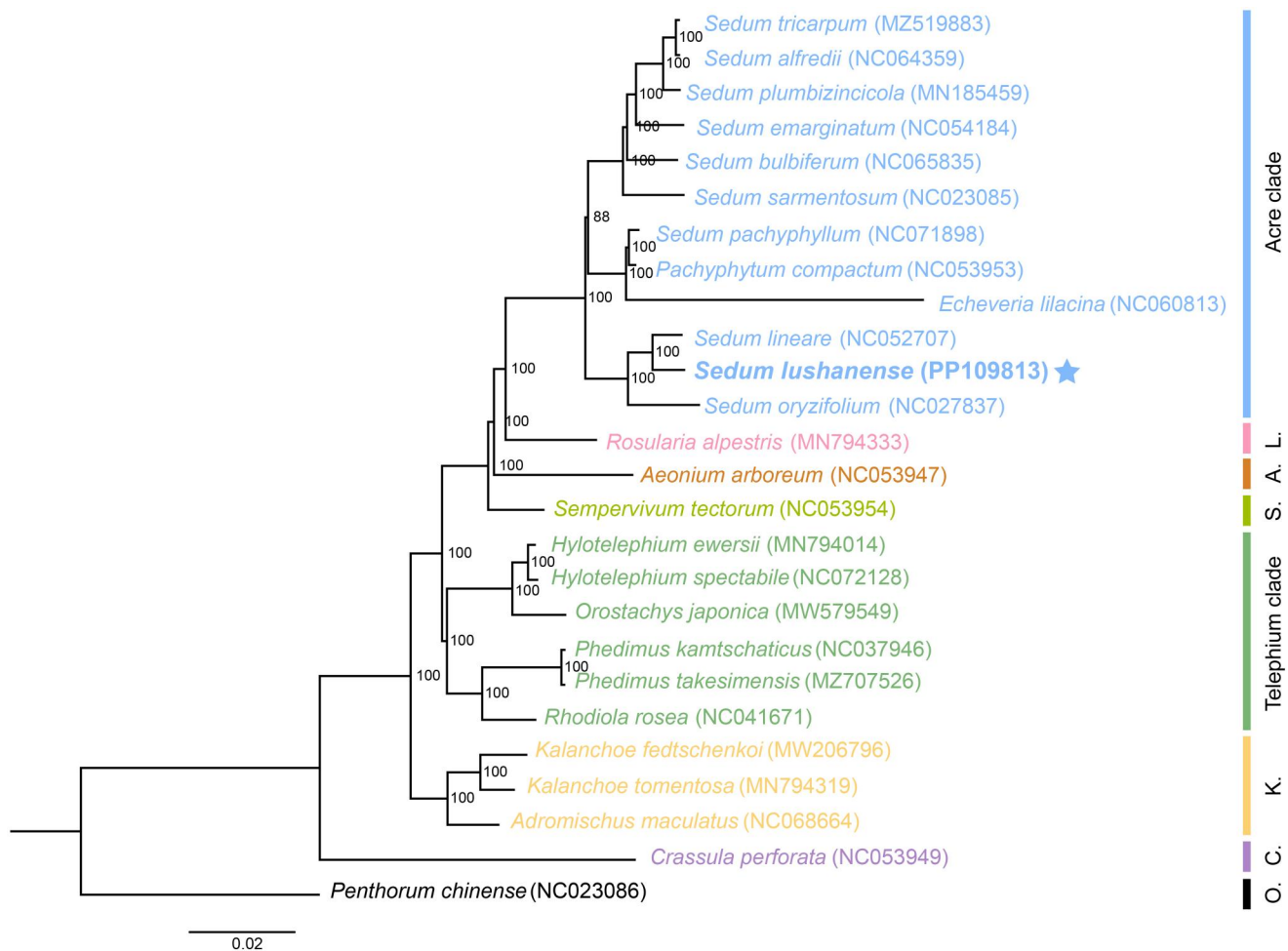


Figure 3. Phylogenetic tree, based on complete chloroplast sequences, made using the maximum likelihood method, including 25 species of the Crassulaceae family. Some clades are represented by letters, including O. (the outgroup clade); C. (the Crassula clade); K. (the Kalanchoe clade); S. (the Sempervivum clade); A. (the Aeonium clade); L. (the leucosedum clade). The following sequences were used: *Sedum tricarpum* (MZ519883; Chen et al. 2022), *Sedum alfredii* (NC064359; Deng et al. 2023), *Sedum plumbizincicola* (MN185459; Deng et al. 2023), *Sedum emarginatum* (NC054184; Chang et al. 2020), *Sedum bulbiferum* (NC065835; Deng et al. 2023), *Sedum sarmentosum* (NC023085; Deng et al. 2023), *Sedum pachyphyllum* (NC071898; Liu et al. 2023), *pachyphytum compactum* (NC053953; Liu et al. 2023), *echeveria lilacina* (NC060813; Liu et al. 2023), *Sedum lineare* (NC052707; Deng et al. 2023), *Sedum lushanense* (PP109813), *Sedum oryzifolium* (NC027837; Deng et al. 2023), *Rosularia alpestris* (MN794333; Deng et al. 2023), *Aeonium arboreum* (NC053947; Chen et al. 2022), *Sempervivum tectorum* (NC053954; Chen et al. 2022), *Hylotelephium ewersii* (MN794014; Liu et al. 2023), *Hylotelephium spectabile* (NC072128; Liu et al. 2023), *Orostachys japonica* (MW579549; Deng et al. 2023), *Phedimus kamtschaticus* (NC037946; Liu et al. 2023), *Phedimus takesimensis* (MZ707526; Liu et al. 2023), *Rhodiola rosea* (NC041671; Liu et al. 2023), *Kalanchoe fedtschenkoi* (MW206796; Liu et al. 2023), *Kalanchoe tomentosa* (MN794319; Chen et al. 2022), *Adromischus maculatus* (NC068664; Chen et al. 2022), *Crassula perforata* (NC053949), *Penthorum chinense* (NC023086; Dong et al. 2013).

region to Asia, where they subsequently diversified (Liu et al. 2023). *S. lushanense* is primarily distributed in the Lushan Mountains in China, where it has adapted to the alpine environment. A similar alpine adaptive evolutionary pattern has been observed in other plants within the Crassulaceae family. For instance, the genus *Rhodiola* has been shown to undergo rapid radiation on the Qinghai-Tibet Plateau and its neighboring regions (Zhang et al. 2014). The phylogenetic tree indicated that *S. lushanense* clustered closely with *S. lineare*, with strong bootstrap support (100%). *S. lushanense*, *S. lineare*, and *S. oryzifolium* formed monophyletic groups in the Acre clade. This genetic information would be considerably significant for advancing conservation efforts and facilitating phylogenomic studies on *S. lushanense*. In future, we plan to expand the chloroplast genome data to include additional *Sedum* species to enhance our understanding of the genus. Our findings underscored the significance of the ongoing exploration and genetic characterization of plant biodiversity.

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Ethical approval

The material in the article does not present any ethical conflicts. The species in this study was not collected from a natural reserve, so no special permits or licenses were required. This work adheres to the Regulations of the People's Republic of China on the Protection of Wild Plants, the International Union for Conservation of Nature (IUCN) policies on research involving species at risk of extinction, the Convention on Biological Diversity, and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Authors' contributions

XXZ designed the research and revised the manuscript. TJL identified and collected the sample. EDY and JZ conducted the experiment. EDY

and ZYL analyzed the data. EDY and JZ wrote the manuscript. All authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data supporting the findings of this study are available in GenBank (<https://www.ncbi.nlm.nih.gov/>) under accession no. PP109813. The associated BioProject, SRA, and Biosample numbers are PRJNA1064019, SRX23261774, and SAMN39415832, respectively.

References

- Carrillo-Reyes P, Sosa V, Mort ME. 2009. Molecular phylogeny of the *Acre* clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Mol Phylogenet Evol.* 53(1):267–276. doi:10.1016/j.ympev.2009.05.022.
- Chang H, Tang Y, Xu X. 2020. The complete chloroplast genome of *Sedum emarginatum* (Crassulaceae). *Mitochondrial DNA Part B Resour.* 5(3):3082–3083. doi:10.1080/23802359.2020.1797567.
- Chen J, Zhou H, Gong W. 2022. The complete chloroplast genome of *Sedum tricarpum* Makino. (Crassulaceae). *Mitochondrial DNA Part B Resour.* 7(1):15–16. doi:10.1080/23802359.2021.2005489.
- Deng ZJ, Huang KR, Xie P, Xie SS, Zhang NY, Yin HB, Ping M, Wang Y. 2023. The complete chloroplast genome sequence of *Sedum bulbiferum* (Crassulaceae). *Mitochondrial DNA Part B Resour.* 8(5):598–602. doi:10.1080/23802359.2022.2160220.
- Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Res.* 45(4):e18. doi:10.1093/nar/gkw955.
- Dong WP, Xu C, Cheng T, Lin K, Zhou SL. 2013. Sequencing angiosperm plastid genomes made easy: A complete set of universal primers and a case study on the phylogeny of Saxifragales. *Genome Biol Evol.* 5(5): 989–997. doi:10.1093/gbe/evt063.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem Bull.* 19:11–15.
- Feng C, Zhang J, Huang HH, 1. 2023. *Parallel situ* conservation: a new plant conservation strategy to integrate *in situ* and *ex situ* conservation of plants. *Biodiv Sci.* 31(9):23184. doi:10.17520/biods.2023184.
- Fu KT, Ohba H. 2001. Crassulaceae. In: Wu ZY, Raven PH, editors. *Flora of China* (Vol. 8). Beijing: Science Press, St. Louis: China and Missouri Botanical Garden Press, ; p. 202–268.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30(14):3059–3066. doi:10.1093/nar/gkf436.
- Kim SH, Kim SC. 2020. Plastid genome of stonecrop *Hylotelephium verticillatum* (Sedoideae; Crassulaceae): insight into structure and phylogenetic position. *Mitochondrial DNA Part B Resour.* 5(3):2729–2731. doi:10.1080/23802359.2020.1788464.
- Lavi B, Levy Karin E, Pupko T, Hazkani-Covo E. 2018. The prevalence and evolutionary conservation of inverted repeats in proteobacteria. *Genome Biol Evol.* 10(3):918–927. doi:10.1093/gbe/evy044.
- Letunic I, Bork P. 2021. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 49(W1): W293–W296. doi:10.1093/nar/gkab301.
- Liang TJ, Tang S, Li XH, Chen BH, Zhang Y, Nie JB, Nie H. 2014. Investigation of some plant resources named for Lushanensis or Gulingensis. *Chin Wild Plant Resour.* 33:64–67.
- Liu XY, Zhang DQ, Zhang JQ. 2023. Plastomic data shed new light on the phylogeny, biogeography, and character evolution of the family Crassulaceae. *J Sytem Evol.* 61(6):990–1003. doi:10.1111/jse.13003.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 32(1):268–274. doi:10.1093/molbev/msu300.
- Ni Y, Li JL, Zhang C, Liu C. 2023. Generating sequencing depth and coverage map for organelle genomes. *Protocols.io.* doi:10.17504/protocols.io.4r3l27jkg1y/v1.
- Shi L, Chen H, Jiang M, Wang L, Wu X, Huang L, Liu C. 2019. CPGAVAS2, an integrated plastome sequence annotator and analyzer. *Nucleic Acids Res.* 47(W1):W65–W73. doi:10.1093/nar/gkz345.
- Stephenson R. 1994. *Sedum: Cultivated Stonecrops*. Portland: Timber Press; p. 355.
- Thiede J, Eggli U. 2007. Crassulaceae. In: Kubitzki K, editor. *Families and genera of vascular plants IX*. Berlin, Heidelberg: Springer, pp p. 83–118. doi:10.1007/978-3-540-32219-1_12.
- Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq-versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 45(W1):W6–W11. doi:10.1093/nar/gkx391.
- Zhang JQ, Meng SY, Allen GA, Wen J, Rao GY. 2014. Rapid radiation and dispersal out of the Qinghai-Tibetan Plateau of an alpine plant lineage *Rhodiola* (Crassulaceae). *Mol Phylogenet Evol.* 77:147–158. doi:10.1016/j.ympev.2014.04.013.