

MOLECULAR ORGANIZATION OF THE CHLOROPLAST GENOME AND TAXONOMIC POSITION OF *STELLARIA DICHOTOMA* VAR. *LANCEOLATE*

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Stellaria dichotoma L. var. *lanceolata* Bunge is a typical rare medicinal plant commonly used in therapeutic formulations. To reveal the structural arrangements and variation of complete chloroplast genomes between *S. dichotoma* var. *lanceolata* and its related species is of great significance for the study of its evolutionary status. In this study, evolutionary relationships between *S. dichotoma* var. *lanceolata* and its related species of Caryophyllaceae were documented based on the complete chloroplast genome sequence of *S. dichotoma* var. *lanceolata*. The result showed that the whole circular genome of *S. dichotoma* var. *lanceolata* was 150,461 bp in length, annotated 129 genes, possessing RSCU of 21 types of amino acids and 64 codons encoding. By comparing and analyzing the SSR and variation region of the chloroplast gene of *S. dichotoma* var. *lanceolata* and its related genus *pseudostellaria*, we found that the divergent regions of *trnk-rps16*, *atpH-atpI*, *rpoC1-rpoB*, *rbcL-accD*, *trnS-trnG*, *psaA-ycf3*, *trnV-trnM*, *ycf4-cemA*, *petL-petG*, *trnL-ccsA*, *ndhF*, *ndhA* and *ycf1* fragments were highly obvious, which could be used as DNA barcodes for the taxonomic evidence of *S. dichotoma* var. *Lanceolata* and *Pseudostellaria* in Caryophyllaceae. A maximum likelihood (ML) phylogenetic tree elucidated that *S. dichotoma* var. *lanceolata* was closely related to *pseudostellaria*, and cluster into a branch with *Cerastium*. Our results lay a robust foundation for future phylogenetic and evolutionary status of *S. dichotoma* var. *lanceolata* and among relatives within Caryophyllaceae.

Key words: Complete chloroplast genome; *Stellaria dichotoma* var. *lanceolata*; Caryophyllaceae; *Pseudostellaria*; phylogenetic analysis.

МОЛЕКУЛЯРНА ОРГАНІЗАЦІЯ
ХЛОРОПЛАСТНОГО ГЕНОМУ
ТАКСОНОМІЧНЕ ПОЛОЖЕННЯ *STELLARIA*
DICHOTOMA VAR. *LANCEOLATE*

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Stellaria dichotoma L. var. *lanceolata* Bunge – це типова рідкісна лікарська рослина, яку зазвичай використовують у складі терапевтичних препаратів. Виявлення структурних особливостей і відмінностей повних хлоропластних геномів *S. dichotoma* var. *lanceolata* від споріднених видів є надзвичайно важливим для вивчення його еволюційного статусу. У цьому дослідженні було задокументовано еволюційні взаємовідносини між *S. dichotoma* var. *lanceolata* та спорідненими видами Caryophyllaceae на основі послідовності повного хлоропластного геному *S. dichotoma* var. *lanceolata*. Результат продемонстрував, що повний циклічний геном *S. dichotoma* var. *lanceolata* мав довжину 150 461 п.н., 129 генів, відносну частоту використання синонімічного кодону (RSCU) 21 типів амінокислот і 64 кодоні. Шляхом порівняння й аналізу простих повторюваних послідовностей (SSR) і ділянки мінливості хлоропластного гену *S. dichotoma* var. *lanceolata* і його спорідненого роду *Pseudostellaria* ми виявили, що дивергуючі області *trnk-rps16*, *atpH-atpI*, *rpoC1-rpoB*, *rbcL-accD*, *trnS-trnG*, *psaA-ycf3*, *trnV-trnM*, *ycf4-cemA*, *petL-petG*, *trnL-ccsA*, *ndhF*, *ndhA* та *ycf1* фрагментів були надзвичайно очевидними, що можна використати як ДНК-штрихи для таксономічного доведення *S. dichotoma* var. *Lanceolata* та *Pseudostellaria* в Caryophyllaceae. Філогенетичне дерево максимальної правдоподібності показало, що *S. dichotoma* var. *lanceolata* тісно пов’язана з *Pseudostellaria* і кластером на гілці із *Cerastium*. Наши результати закладають міцне підґрунтя для майбутнього філогенетичного і еволюційного статусу *S. dichotoma* var. *lanceolata* та положення серед Caryophyllaceae.

Ключові слова: повний хлоропластний геном; *Stellaria dichotoma* var. *lanceolata*; Caryophyllaceae; *Pseudostellaria*; філогенетичний аналіз.

REFERENCES

- Altschul SF, Madden TL, Schäffer AA, Zhang JH, Zhang Z, Miller W et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl Acids Res 25:3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
- Alzahrani DA (2021) Complete chloroplast genome of *Abutilon fruticosum*: Genome structure, comparative and phylogenetic analysis. Plants (Basel, Switzerland) 10(2):270. <https://doi.org/10.3390/PLANTS10020270>.
- Amiryousefi A, Hyvonen J, Poczai P (2018) IRscope: an online program to visualize the junction sites of chloroplast genomes. Bioinformatics 34:3030–3031. <https://doi.org/10.1093/bioinformatics/bty220>.
- Androsiuk P, Jastrzebski JP, Paukszt T, Makowczen-

- ko K, Okouski A, Pszczotkowska A et al (2020) Evolutionary dynamics of the chloroplast genome sequences of six *Colobanthus* species. *Sci Rep* 10:11522. <https://doi.org/10.1038/s41598-020-68563-5>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Computat Biol* 19(5):455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Beier S, Thiel T, Münch T, Scholz U, Mascher M (2017) MISA-web: a web server for microsatellite prediction. *Bioinformatic* 33:2583–2585. <https://doi.org/10.1093/bioinformatics/btx198>.
- Benson G (1999) Tandem repeats finder: A program to analyze DNA sequences. *Nucl Acids Res* 27:573–580. <https://doi.org/10.1093/nar/27.2.573>.
- Byng JW, Chase MW, Christenhusz MJ, Fay MF, Judd WS, Mabberley DJ et al (2016) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. *Botan J Linnean Soc* 181:1–20. <https://doi.org/10.1111/boj.12385>.
- Chen XB, Meng SY, Zhang XF, Han YB, Liu QR (2014) Numerical taxonomic analysis of *Stellaria* and *Pseudostellaria* (Caryophyllaceae) (in Chinese). *Chinese Bull Bot* 49(04):432–439. <https://doi.org/10.3724/SP.J.1259.2014.00432>.
- Chen YF, Kuo PC, Chan HH, Kuo IJ, Lin FW, Su CR et al (2010) Beta-Carboline Alkaloids from *Stellaria dichotoma* var. *lanceolata* and their anti-inflammatory activity. *J Nat Prod*, 73:1993–1998. <https://doi.org/ir.cmu.edu.tw/ir/handle/310903500/42285>.
- Cui N, Liao BS, Liang CL, Li SF, Zhang H, Xu J et al (2020) Complete chloroplast genome of *Salvia plebeia*: organization, specific barcode and phylogenetic analysis. *Chinese J Nat Med* 18(08):563–572. [https://doi.org/10.1016/S1875-5364\(20\)30068-6](https://doi.org/10.1016/S1875-5364(20)30068-6).
- Dong WP, Liu H, Xu C, Zuo YJ, Chen ZJ, Zhou SL (2014) A chloroplast genomic strategy for designing taxon specific DNA mini-barcodes: A case study on ginsengs. *BMC Genet* 15:138. <https://doi.org/10.1186/s12863-014-0138-z>.
- Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I (2004). VISTA: Computational tools for comparative genomics. *Nucl Acids Res* 32:W273–279. <https://doi.org/10.1093/nar/gkh458>.
- Gao CW, Wu CH, Zhang Q, Zhao X, Wu MX, Chen RR et al (2020) Characterization of chloroplast genomes from two *Salvia* medicinal plants and gene transfer among their mitochondrial and chloroplast genomes. *Frontiers in genetics*, 11:574962. <https://doi.org/10.3389/fgene.2020.574962>.
- Greiner S, Lehwark P, Bock R. 2019. Organellar GenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucl Acids Res* 47:W59–W64. <https://doi.org/10.1093/nar/gkz238>.
- Guo M, Xu Y, Ren L, He S, Pang AX (2018) A systematic study on DNA barcoding of medicinally important genus *Epimedium* L. (Berberidaceae). *Genes* 9(12):637. <https://doi.org/10.3390/genes9120637>.
- Hollingsworth PM, Li DZ, van der BM, Twyford AD (2016). Telling plant species apart with DNA: from barcodes to genomes. *Philos Trans R Soc Lond B Biol Sci* 371(1702):201503. <https://doi.org/10.1098/rstb.2015.0338>.
- Jansen RK, Raubeson LA, Boore JL, dePamphilis CW, Chumley TW, Haberle RC et al (2005) Methods for obtaining and analyzing whole chloroplast genome sequences. *Methods Enzymol* 395:348–384. [https://doi.org/10.1016/S0076-6879\(05\)95020-9](https://doi.org/10.1016/S0076-6879(05)95020-9).
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780. <https://doi.org/10.1093/molbev/mst010>.
- Kučera J, Svitok M, Gbúrová ŠE, Mártonfiová L, Lafon PC, Slovák M (2021) Eunuchs or females? Causes and consequences of gynodioecy on morphology, ploidy, and ecology of *Stellaria graminea* L. (Caryophyllaceae). *Front Plant Sci* 12:589093. <https://doi.org/10.3389/FPLS.2021.589093>.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evolut* 35(6):1547–1549. <https://doi.org/10.1093/molbev/msy096>.
- Li B, Lin FR, Huang P, Guo WY, Zheng YQ (2020c) Development of nuclear SSR and chloroplast genome markers in diverse *Liriodendron chinense* germplasm based on low-coverage whole genome sequencing. *Biol Res* 53(6119):e460–e464. <https://doi.org/10.1186/s40659-020-00289-0>.
- Li J, Li HY, Zhi JK, Shen CZ, Yang XS, Xu JC (2017) Codon usage of expansin genes in *Populus trichocarpa*. *Curr Bioinform* 12(5):452–461. <https://doi.org/10.2174/157489361166161008195145>.
- Li P, Yu P, Xia J (2020b) Characterization of the complete chloroplast genome of *Stellaria dichotoma* var. *lanceolata* Bunge, a traditional Chinese medicinal plant. *Mitochondrial DNA Part B* 5(4):3848–3850. <https://doi.org/doi.org/10.1080/23802359.2020.1841578>.
- Li ZK, Song L, Lei Y, Liang WL, Wang H, Peng L (2020a) Advances in biology, chemical constituents and pharmacological activities of *Stellaria dichotoma* var. *lanceolata* (in Chinese). *J Nanjing Univ Tradit Chin Med* 36(1):136–140. <https://doi.org/10.14148/j.issn.1672-0482.2020.0136>.

- Luo C, Li Y, Budhathoki R, Shi J, Yer H, Li X et al (2021) Complete chloroplast genomes of *Impatiens cyanantha* and *Impatiens monticola*: Insights into genome structures, mutational hotspots, comparative and phylogenetic analysis with its congeneric species. *PLoS one* 16(4):e0248182. <https://doi.org/10.1371/JOURNAL.PONE.0248182>.
- Luo K, Ma P, Song JY, Chen KL, Liu YM (2012) Molecular identification of Fritillariae Cirrhosae Bulbus and its adulterants (in Chinese). *World Sci Technol Modern Tradit Chin Med Materia Medica* 14:1153–1158. <https://doi.org/10.11842/wst.2012.1>.
- Maheswari P, Kunhikannan C, Yasodha R (2021) Chloroplast genome analysis of Angiosperms and phylogenetic relationships among Lamiaceae members with particular reference to teak (*Tectona grandis* L.f.). *J Biosci* 46(2):43. <https://doi.org/10.1007/S12038-021-00166-2>.
- Mishra P, Kumar A, Nagireddy A, Mani DN, Shukla AK, Tiwari R et al (2015) DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market. *Plant Biotechnol J* 14:8–21. <https://doi.org/10.1111/pbi.12419>.
- National Pharmacopoeia Committee (2015). *Pharmacopoeia of the People's Republic of China* (in Chinese). Beijing: China Medical Science and Technology Press, 317 p.
- Njuguna AW, Li ZZ, Saina JK, Munywoki JM, Gichira AW, Gituru RW et al (2019) Comparative analyses of the complete chloroplast genomes of *Nymphoides* and *Menyanthes* species (menyanthaceae). *Aquatic Botany* 156:73–81. <https://doi.org/10.1016/j.aquabot.2019.05.001>.
- Park I, Yang S, Song JH, Moon BC (2020) Dissection for floral micromorphology and plastid genome of valuable medicinal borages *Arnebia* and *Lithospermum* (Boraginaceae). *Front Plant Sci* 11:606463. <https://doi.org/10.3389/FPLS.2020.606463>.
- Parks M, Cronn R, Liston A (2009) Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology* 7:84. <https://doi.org/10.1186/1741-7007-7-84>.
- Peng J, Zhao YL, Dong M, Liu SQ, Hu ZY, Zhong XF et al (2021) Exploring the evolutionary characteristics between cultivated tea and its wild relatives using complete chloroplast genomes. *BMC Ecol Evolut*, 21(1):71–71. <https://doi.org/10.1186/S12862-021-01800-1>.
- Rozas J, Ferrermata A, Sánchezdelbarrio JC, Guirao-rico S, Librado P, Ramosonsins SE, Sánchezgracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large datasets. *Mol Biol Evolut* 34:3299–3302. <https://doi.org/10.1093/molbev/msx248>.
- Sharples MT (2019) Taxonomic observations within *Stellaria* (Caryophyllaceae): Insights from ecology, geography, morphology, and phylogeny suggest widespread parallelism in starworts and erode previous infrageneric classifications. *Systemat Bot* 44(4):877–886. <https://doi.org/10.1600/036364419X15710776741459>.
- Sharples MT, Bentz PC, Manzitto-Tripp EA (2021) Evolution of apetalous in the cosmopolitan genus *Stellaria*. *Amer J Bot* 108(5):869–882. <https://doi.org/10.1002/AJB2.1650>.
- Sharples MT, Tripp EA (2019) Phylogenetic relationships within and delimitation of the cosmopolitan flowering plant genus *Stellaria* L. (Caryophyllaceae): Core stars and fallen stars. *Systemat Bot* 44(4):857–876. <https://doi.org/10.1600/036364419X15710776741440>.
- Stefanova P, Taseva M, Georgieva T, Gotcheva V, Angelov A (2013). A modified CTAB method for DNA extraction from soybean and meat products. *Biotechnol Biotec Eq* 27(3):3803–3810. <https://doi.org/10.5504/BBEQ.2013.0026>.
- Van Do T, Xu B, Gao XF (2021) Molecular phylogeny and character evolution of *Flemingia* (Leguminosae, Papilionoideae, Phaseoleae, Cajaninae) inferred from three cpDNA and nrITS sequence data. *Plant System Evolut* 307:30. <https://doi.org/10.1007/S00606-021-01749-0>.
- Wei F, Tang DF, Wei KH, Qin F, Li LX, Lin Y et al (2020) The complete chloroplast genome sequence of the medicinal plant *Sophora tonkinensis*. *Scientific reports* 10:12473. <https://doi.org/10.1038/s41598-020-69549-z>.
- Wei L, De Craene LR (2019) What is the nature of petals in Caryophyllaceae? Developmental evidence clarifies their evolutionary origin. *Ann Bot* 124:1–15. <https://doi.org/10.1093/aob/mcz075>.
- Wyman SK, Jansen RK, Boore JL (2004) Automatic annotation of organelar genomes with DOGMA. *Bioinformatics* 20:3252–3255.
- Xu C, Dong W, Li W, Lu Y, Xie X, Jin X et al (2017). Comparative analysis of six *Lagerstroemia* complete chloroplast genomes. *Front Plant Sci* 8:15. <https://doi.org/10.1093/bioinformatics/bth352>.
- Yang HS, Li XP, Liu DJ, Chen XB, Li FH, Qi XL et al (2016) Genetic diversity and population structure of the endangered medicinal plant *Phelldendron amurense* in China revealed by SSR markers. *Biochem Systemat Ecol* 66:286–292. <https://doi.org/10.1016/j.bse.2016.04.018>.
- Zalapa JE, Cuevas H, Zhu H, Steffan S, Senalik D, Zeldin E et al (2012) Using next-generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. *Amer J Bot* 99:193–208. <https://doi.org/10.3732/ajb.1100394>.

- Zhang WJ, Cao Z, Xie ZC, Lang DY, Zhou L, Chu YK et al (2017). Effect of water stress on roots biomass and secondary metabolites in the medicinal plant *Stellaria dichotoma* L. var. *lanceolata* Bge. *Scientia Horticulturae* 224:280–285. <https://doi.org/10.1016/j.scienta.2017.06.030>.
- Zhang Y, Wang ZF, Guo YN, Chen S, Xu XY, Wang RJ (2021) Complete chloroplast genomes of *Leptodermis scabrida* complex: Comparative genomic analyses and phylogenetic relationships. *Gene* 791:145715. <https://doi.org/10.1016/J.GENE.2021.145715>.
- Zheng G, Wei LL, Ma L, Wu ZQ, Gu CH, Chen K (2020) Comparative analyses of chloroplast genomes from 13 *Lagerstroemia* (Lythraceae) species: identification of highly divergent regions and inference of phylogenetic relationships. *Plant Mol Biol* 102(6):659–676. <https://doi.org/10.1007/S11103-020-00972-6>.
- Zhou L, Lang DY, Zhang WJ, Wang JH, Gao XJ, Wu XL et al (2019) Physiological mechanisms of salt and drought-induced stress effects on root biomass and secondary metabolites in *Stellaria dichotoma*. *Inter J Agricult Biol* 22:1285–1292. <https://doi.org/10.17957/IJAB/15.1200>.
- Zhu S, Liu QD, He JY, Nakajima N, Samarakoon SP, Swe S et al (2021) Genetic identification of medicinally used *Salacia* species by nrDNA ITS sequences and a PCR-RFLP assay for authentication of *Salacia*-related health foods. *J Ethnopharmacol* 274:113909. <https://doi.org/10.1016/J.JEP.2021.113909>.

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