


The complete plastome of *Cynanchum rostellatum* (Apocynaceae), an indigenous plant in Korea

Sae Hyun Lee^{a*}, Woojong Jang^{a,b*}, Eunbi Kim^{a*}, Jiseok Kim^a, Haiguang Gong^{a,c}, Jong-Soo Kang^a, Hyeonah Shim^a, Jee Young Park^a and Tae-Jin Yang^a 

^aDepartment of Agriculture, Forestry and Bioresources, Plant Genomics and Breeding Institute, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea; ^bNational Institute of Horticultural and Herbal Science, RDA, Eumseong, Republic of Korea; ^cKey Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement, Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, PR China

ABSTRACT

The climbing plant *Cynanchum rostellatum* (Turcz.) Liede & Khanum is widely distributed throughout Korea and Northeast Asia as a member of the Apocynaceae family. Although this plant has a high value in medicinal and industrial purposes, genetic research on this plant is insufficient. This study announces the complete plastid genome (plastome) sequence of *C. rostellatum* with 663× mean coverage, which was assembled using 763 Mbp short-read data generated by the Illumina HiSeq X platform. The *C. rostellatum* plastome was 158,018 bp in length and displayed the typical quadripartite structure composed of the large single-copy (LSC) region (89,058 bp), the small single-copy (SSC) region (18,718 bp), and a pair of inverted repeat (IR) regions (25,116 bp). A total of 129 genes have been annotated, including 84 protein-coding genes, 37 transfer RNA genes, and eight ribosomal RNA genes. Phylogenetic analysis indicated the genus *Cynanchum* including 12 *Cynanchum* plastome sequences, was monophyletic and was located within the sub-family Asclepiadoideae. Two *C. rostellatum* plastomes, including the plastome assembled in this study, formed a subclade and were sister to the *C. thesioides* plastome, whereas the other *C. rostellatum*, which was previously reported one, was located within the clade of *C. wilfordii* and *C. bungei*.

ARTICLE HISTORY

Received 14 July 2022
Accepted 13 November 2022

KEYWORDS



Plastid genome;
phylogenetic analysis;
Cynanchum; *Cynanchum rostellatum*

Cynanchum rostellatum (Turcz.) Liede & Khanum (2016) is a perennial plant belonging to the Apocynaceae family and Asclepiadoideae sub-family and has white milky sap and climbing vines (Khanum et al. 2016) (Figure 1). The root of this species is widespread and has many buds for vegetative propagation. In addition, their seeds have feathers for propagation (Kim et al. 2014). This species previously belonged to the genus *Metaplexis* and was recognized as *Metaplexis japonica* (Thunb.) Makino (1903). However, the genus *Metaplexis* was recently merged with the genus *Cynanchum* based on molecular evidence of several barcoding regions, including plastid and nuclear regions (Khanum et al. 2016). The *C. rostellatum* has traditionally been used as a medicinal plant for erectile dysfunction (Wei et al. 2019). Additional functions such as antibacterial, antioxidant, and nerve cell protection effects have also been reported (Jamarkattel-Pandit and Kim 2019; Wei et al. 2019). Moreover, the feathers on the seeds can separate oil from water, increasing their industrial value (Wang et al. 2019). Although *C. rostellatum* is morphologically similar to other *Cynanchum* species with climbing vines, such as *C. wilfordii* (Kim et al. 2014). This

species can be distinguished from other species by having an elongated stigma and purple furry petals in flower morphology (Nam and Chung 2018). However, when flowers fall, *C. rostellatum* is frequently misidentified as *C. wilfordii*, one of the most famous medicinal plants in Korea, especially during the harvesting season (Kim et al. 2014).

Therefore, molecular data of this species are still required for species authentication between *C. rostellatum* and other *Cynanchum* species. This study provides information regarding the plastome evolution and intra-species diversity of *C. rostellatum*, which is essential for further species authentication and evolutionary research.

The leaf material of *C. rostellatum* was collected from Hoengseong-gun, Kangwon Province, South Korea (37° 29' 40.10783"N, 128° 1' 29.53267"E), and the specimen was deposited in the T.B.Lee Herbarium of Seoul National University (<http://arbor.snu.ac.kr/eng/>, Sook-Hyang Kim, jaderei@snu.ac.kr) under the voucher number SNUA00057640. Genomic DNA was extracted from newly sprouted young leaves using the GeneAll Exgene plant midi kit (Geneall Biotechnology Ltd., Seoul, South Korea). Pair-end library

CONTACT Tae-Jin Yang  tjyang@snu.ac.kr  Department of Agriculture, Forestry and Bioresources, Plant Genomics and Breeding Institute, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

*These authors contributed equally to this work.

 Supplemental data for this article is available online at <https://doi.org/10.1080/23802359.2022.2148489>

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

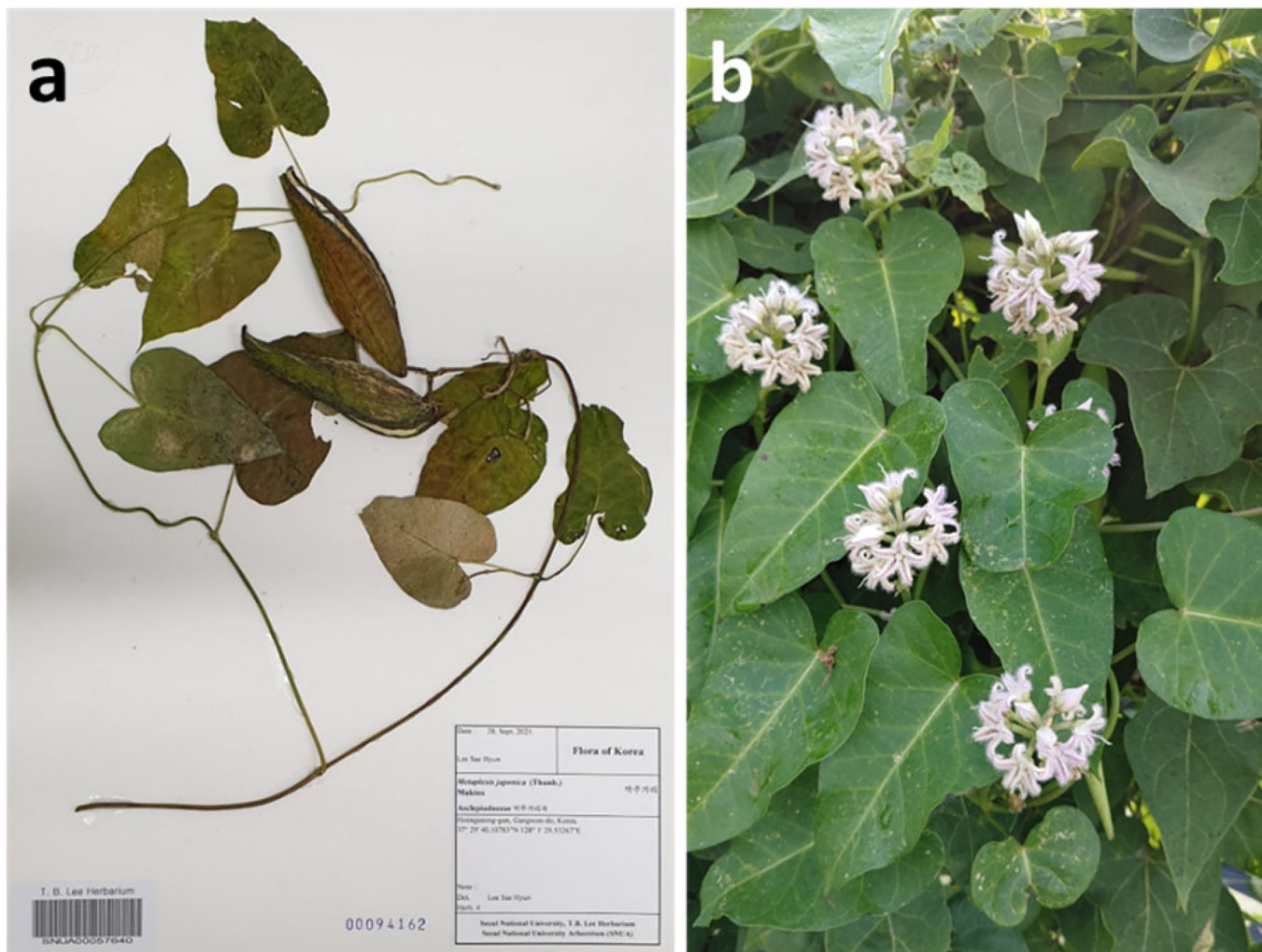


Figure 1. The specimen and morphology of *C. rostellatum*. (a) The specimen of *C. rostellatum* (voucher number: SNUA00057640). (b) The shape of flowers and leaves of *C. rostellatum*.

construction was conducted using HiSeq X Reagent Kit (Illumina, San Diego, CA), and the insert size of 550 bp. Sequencing was conducted on the Illumina HiSeq X platform (Lab genomics LLC, Seongnam, South Korea). The total read length of raw data was 763,415,230 bp. The following information describes how the complete plastome was assembled using the dnaLCW method (Kim et al. 2015). *De novo* assembly was conducted with trimmed raw data using CLC Genomics Workbench (version 10.0.3, CLC Inc., Aarhus, Denmark). Among assembled contigs, plastome sequences were retrieved, ordered, and merged into a single sequence, using the *C. wilfordii* plastome sequence as the reference (Lee et al. 2022). Remained gaps were closed with SOAP Gapcloser (<http://soap.genomics.org.cn>). The average and minimum read mapping depth of assembled genome were 663.34 \times and 80 \times , respectively (Figure S1). Gene annotation of the plastome sequence was conducted using GeSeq (Tillich et al. 2017) and then manually curated. The plastome map was drawn using the CPGView program (<http://www.1kmpg.cn/cpgview>).

For phylogenetic analysis, a total of 38 plastome sequences were downloaded from the NCBI GenBank. Members of the subfamily Rauvolfioideae, *Rhazya stricta* (KJ123753), and *Catharanthus roseus* (NC 021423) were used as an outgroup.

From 39 plastome sequences, 50 co-existing protein-coding genes were selected and merged from each plastome sequence. Sequences were aligned with the MAFFT (Katoh and Standley 2013) and trimmed using Gblocks (Talavera and Castresana 2007). The ModelFinder option of the IQ-TREE program figured out the best tree model, and the TVM + F + I + G4 substitution model was selected for the analysis (Nguyen et al. 2015). A maximum-likelihood (ML) tree was constructed using the IQ-TREE (Nguyen et al. 2015) with 1000 bootstrap replicates. Using in-house Python code, sequence variations were counted from the aligned sequences of three *C. rostellatum*.

The plastome of *C. rostellatum* was 158,018 bp in length, organized into a quadripartite structure, including large single-copy (LSC) (89,058 bp), small single-copy (SSC) (18,728 bp), and inverted repeat (IR) (25,116 bp) regions. The GC contents of the LSC, SSC, and IR regions were 36.3%, 32.2%, and 43.1%, respectively. A total of 129 genes were annotated, consisting of 84 protein-coding genes, 37 tRNA genes, and eight rRNA genes (Figure 2).

Our phylogenetic analysis indicated that the genus *Cynanchum* was monophyletic, and the clade of *Cynanchum* included four subclades (Figure 3). Two *C. rostellatum* plastomes (OM177668 and ON882042) formed a subclade with

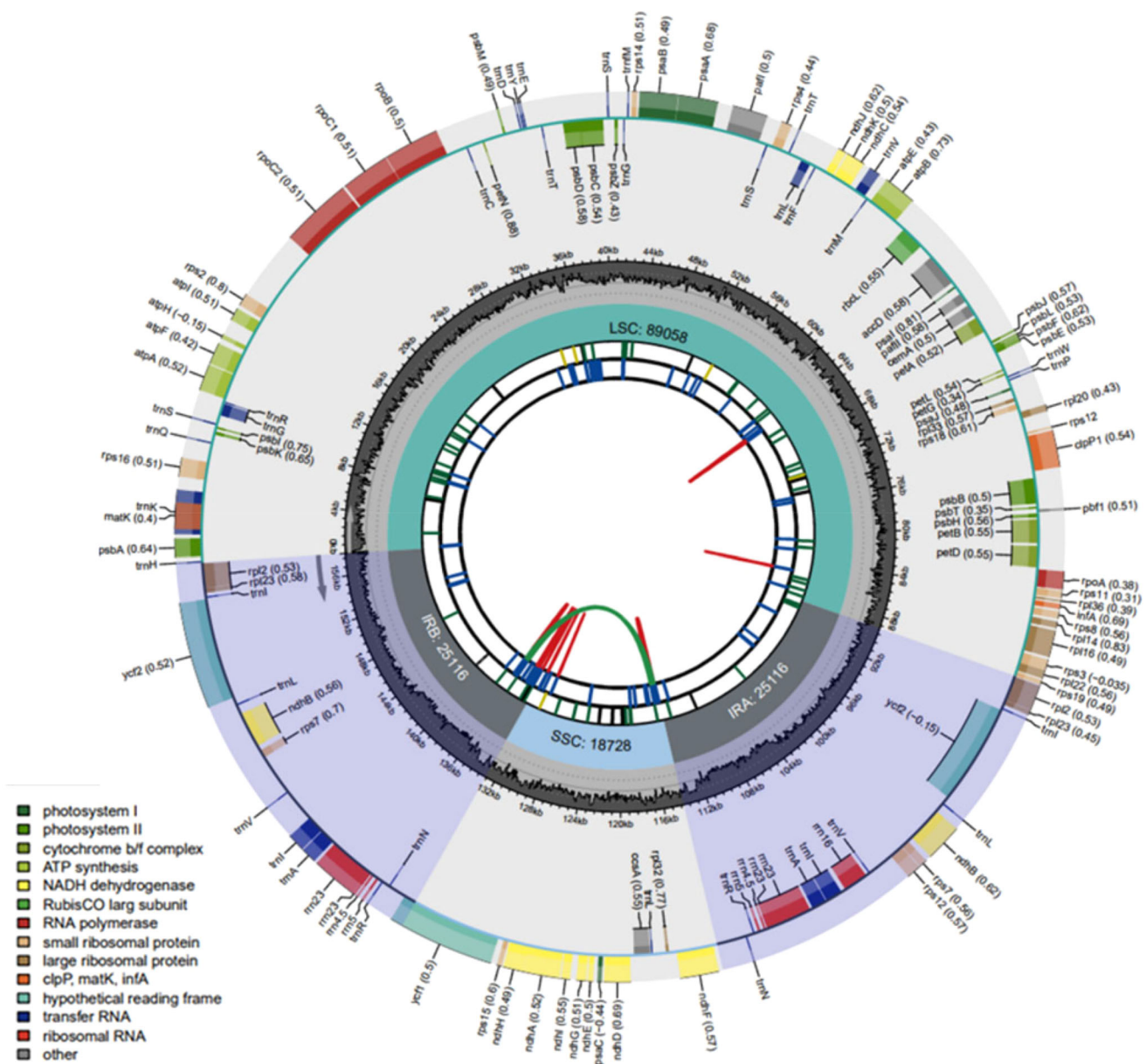


Figure 2. Plastome genome map of *C. rostellatum*. From the inner circle, the first circle depicts distributed repeats connected by red (forward direction) and green (reverse direction) arcs, respectively. The following circle displays tandem repeats denoted by short blue bars. The sequences of microsatellites are depicted as short green bars. The fourth circle displays the sizes of LCS, SSC, and IR. The fifth circle illustrates the distribution of GC contents along the plastome (dark grey: GC contents, light grey: background). The sixth circle displays the genes with colored boxes. The outer and inner colored boxes present transcribed clockwise and counter-clockwise genes, respectively.

the *C. thesioides* plastome (MW864598), and the subclade was sister to other *Cynanchum* plastomes. The *C. chinense* plastome was sister to the clade of *C. wilfordii*, *C. bungei*, *C. auriculatum*, and one *C. rostellatum* (OL689165). The plastomes of *C. wilfordii* and *C. auriculatum* formed independent subclades. However, one *C. rostellatum* plastome (OL689165) formed a subclade with the *C. bungei* plastome. The conflict positions among three *C. rostellatum* plastomes in the phylogeny were also supported by the sequence variation analysis. The result showed that 34 variations (16 SNPs and 18 InDels) were found between two plastomes, OM177668 and ON882042, whereas 4579 variations (3776 SNPs and 803 InDels) were found between OM177668 and OL689165. The great intra-specific diversity in *C. rostellatum* implies the

necessity of further research for this species in terms of genetic diversity and taxonomic treatment. This study provides fundamental information for further studies on species authentication of *C. rostellatum* and the evolution of the Apocynaceae family.

Ethical approval

This study complies with Seoul National University's Research Ethics Guidelines, relevant institutional, national, and international guidelines and legislation. Permission was not required for sample collection in this study. *C. rostellatum* is widespread in South Korea and is not listed as a threatened or endangered species.

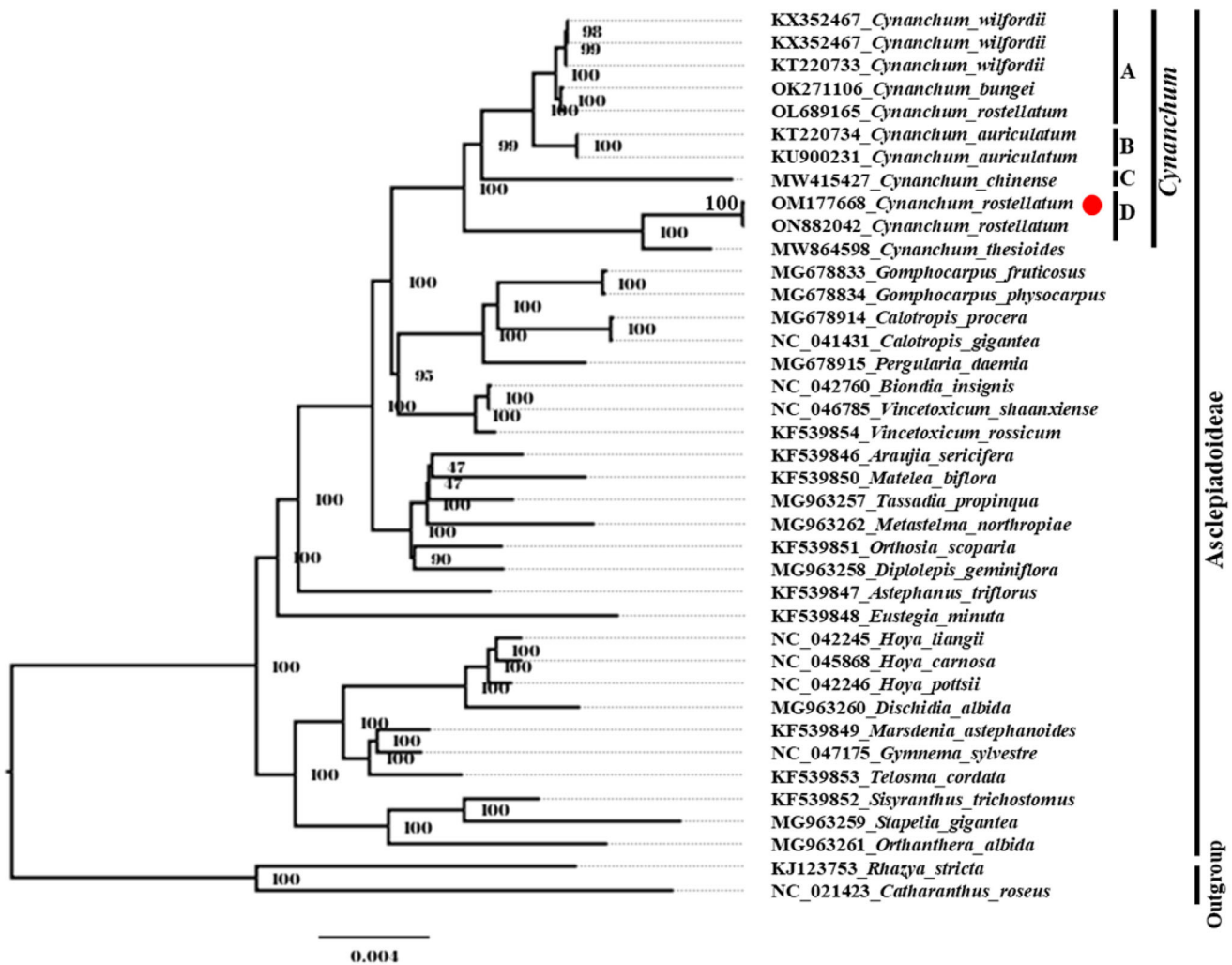


Figure 3. Phylogenetic analysis of *C. rostellatum* with allied species. A phylogenetic tree was constructed using the maximum-likelihood (ML) method with 1000 bootstrap replicates and 50 common protein-coding gene sequences from the plastome. The red dot represents the assembled plastome sequence in this study. The clades of species are represented with black lines. GenBank accession numbers are as follows: *C. wilfordii* KX352467, *C. wilfordii* KT220733 (Park et al. 2016), *C. bungei* OK271106, *C. rostellatum* OL689165 (Pei et al. 2022), *C. auriculatum* KT220734 (Jang et al. 2016), *C. auriculatum* KU900231, *C. chinense* MW415427 (Chen and Zhang 2022), *C. rostellatum* ON882042, *C. thesioides* MW864598, *Gomphocarpus fruticosus* MG678833 (Fishbein et al. 2018), *G. physocarpus* MG678834 (Fishbein et al. 2018), *Calotropis procera* MG678914, *C. gigantea* NC_041431 (Fishbein et al. 2018), *Pergularia daemia* MG678915 (Fishbein et al. 2018), *Biondia insignis* NC_042760 (Guan and Zhang 2019), *Vincetoxicum shaanxiense* NC_046785 (Rao et al. 2018), *V. rossicum* KF539854 (Straub et al. 2013), *Araujia sericifera* KF539846 (Straub et al. 2013), *Matelea biflora* KF539850 (Straub et al. 2013), *Tassadia propinqua* MG963257 (Fishbein et al. 2018), *Metastelma northropiae* MG963262 (Fishbein et al. 2018), *Orthosia scoparia* KF539851 (Straub et al. 2013), *Diplolepis geminiflora* MG963258 (Fishbein et al. 2018), *Astephanus triflorus* KF539847 (Straub et al. 2013), *Eustegia minuta* KF539848 (Wei et al. 2020), *Hoya liangii* NC_042245 (Tan et al. 2018), *H. carnosa* NC_045868 (Wei et al. 2020), *H. pottsii* NC_042246 (Tan et al. 2018), *Dischidia albida* MG963260 (Fishbein et al. 2018), *Marsdenia astephanoides* KF539849 (Straub et al. 2013), *Gymnema sylvestris* NC_047175 (Tan et al. 2018), *Telosma cordata* KF539853 (Straub et al. 2013), *Sisyranthus trichostomus* KF539852 (Straub et al. 2013), *Stapelia gigantea* MG963259 (Fishbein et al. 2018), *Orphanthera albida* MG963261 (Fishbein et al. 2018), *Rhazya stricta* KJ123753, and *Catharanthus roseus* NC_021423 (Ku et al. 2013).

Author contributions

S. H. L., W. J., E. b., and T.-J. Y. planned and designed this research. J. K. and S. H. L. assembled and annotated the plastome sequence. J. K., H. G., J. S. K., H. S., and J. Y. P. collected samples, made specimens, and extracted DNA. S. H. L., W. J., and E. b. wrote the manuscript, J. K., H. G., J. S. K., H. S., and J. Y. P. revised the manuscript.

[No. 2020R1A2C3007885]; Global Visiting Fellowship, BrainKorea 21 Four, Seoul National University, Seoul, Korea; "Cooperative Research Program for Agriculture Science and Technology Development [Project No. PJ017134]" Rural Development Administration, Republic of Korea.

ORCID

Tae-Jin Yang  <http://orcid.org/0000-0002-9676-8801>

Disclosure statement

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

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIT)

Data availability statement

The genome sequence data supporting this study's findings are openly available in GenBank of NCBI (accession number: OM177668). The associated BioProject is PRJNA765723, and this research was conducted as a part of the genome analysis of *Cynanchum* species. SRA and Bio-sample numbers are SRR17333880 and SAMN24429174, respectively.

References

- Chen G, Zhang X. 2022. The complete chloroplast genome of Chinese medicinal herb *Cynanchum chinense* R. Br. (Apocynaceae) and its phylogenetic position. *Mitochondrial DNA Part B*. 7(4):598–599.
- Fishbein M, Livshultz T, Straub SC, Simões AO, Boutte J, McDonnell A, Foote A. 2018. Evolution on the backbone: Apocynaceae phylogenomics and new perspectives on growth forms, flowers, and fruits. *Am J Bot*. 105(3):495–513.
- Fishbein M, Straub SC, Boutte J, Hansen K, Cronn RC, Liston A. 2018. Evolution at the tips: Asclepias phylogenomics and new perspectives on leaf surfaces. *Am J Bot*. 105(3):514–524.
- Guan M, Zhang R. 2019. The complete chloroplast genome of *Biondia insignis* Tsiang (Apocynaceae). *Mitochondrial DNA Part B*. 4(1):280–281.
- Jamarkattel-Pandit N, Kim H. 2019. Neuroprotective effect of *Metaplexis japonica* against in vitro ischemia model. *J Health Allied Sci*. 3(1):51–55.
- Jang W, Kim K-Y, Kim K, Lee S-C, Park H-S, Lee J, Seong RS, Shim YH, Sung SH, Yang T-J. 2016. The complete chloroplast genome sequence of *Cynanchum auriculatum* Royle ex Wight (Apocynaceae). *Mitochondrial DNA Part A*. 27(6):4549–4550.
- Kang S-J, Park H-S, Koo HJ, Park JY, Lee DY, Kang KB, Han SI, Sung SH, Yang T-J. 2018. The complete chloroplast genome sequence of Korean *Lonicera japonica* and intra-species diversity. *Mitochondrial DNA Part B*. 3(2):941–942.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 30(4):772–780.
- Khanum R, Surveswaran S, Meve U, Liede-Schumann S. 2016. *Cynanchum* (Apocynaceae: Asclepiadoideae): a pantropical *Asclepiadoideae* genus revisited. *Taxon*. 65(3):467–486.
- Kim MJ, Kim IJ, Choi SY, Han DH, Kim YH, Lim SC, Kim TJ, Nam SY, Song BH, Oh BU, et al. 2014. Comparison of *Cynanchum wilfordii*, *C. auriculatum*, *Metaplexis japonica* and *Polygonum multiflorum* by morphological characters. *Korean J Med Crop Sci*. 22(2):113–120.
- Kim K, Lee S-C, Lee J, Yu Y, Yang K, Choi B-S, Koh H-J, Waminal NE, Choi H-I, Kim N-H. 2015. Complete chloroplast and ribosomal sequences for 30 accessions elucidate evolution of *Oryza* AA genome species. *Sci Rep*. 5(1):1–13.
- Ku C, Chung W-C, Chen L-L, Kuo C-H. 2013. The complete plastid genome sequence of madagascar periwinkle *Catharanthus roseus* (L.) G. Don: plastid genome evolution, molecular marker identification, and phylogenetic implications in asterids. *PLOS One*. 8(6):e68518.
- Lee SH, Kim J, Park H-S, Koo H, Waminal NE, Pellerin RJ, Shim H, Lee H-O, Kim E, Park JY, et al. 2022. Genome structure and diversity among *Cynanchum wilfordii* accessions. *BMC Plant Biol*. 22(1):4–14.
- Nam B-m, Chung GY. 2018. Taxonomic implications of floral morphology in the subfamily Asclepiadoideae (Apocynaceae s.l.) in Korea. *Korean J Plant Taxon*. 48(3):172–184.
- Nguyen L-T, 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.
- Park H-S, Kim K-Y, Kim K, Lee S-C, Lee J, Seong RS, Shim YH, Sung SH, Yang T-J. 2016. The complete chloroplast genome sequence of an important medicinal plant *Cynanchum wilfordii* (Maxim.) Hemsl. (Apocynaceae). *Mitochondrial DNA Part A*. 27(5):3747–3748.
- Pei L, Shu S, Ji B, Cui N. 2022. Complete sequence of *Cynanchum rostellatum* (Apocynaceae: Asclepiadoideae) chloroplast genome and its phylogenetic analysis. *Mitochondrial DNA Part B*. 7(7):1395–1397.
- Rao H, Wang X-J, Ma J-X, Li Q-E, Li G-L, Zou J-B. 2018. Characterization of the complete chloroplast genome of *Biondia chinensis* (Apocynaceae: Asclepiadoideae: Asclepiadeae), a rare and threatened liana endemic to China. *Mitochondrial DNA Part B*. 3(2):763–764.
- Straub SC, Cronn RC, Edwards C, Fishbein M, Liston A. 2013. Horizontal transfer of DNA from the mitochondrial to the plastid genome and its subsequent evolution in milkweeds (Apocynaceae). *Genome Biol Evol*. 5(10):1872–1885.
- Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol*. 56(4):564–577.
- Tan X-H, Wang J-H, Zhao K-K, Zhu Z-X, Wang H-F. 2018. Complete plastome sequence of *Hoya pottsii* Traill and *Hoya liangii* Tsiang (Apocynaceae). *Mitochondrial DNA Part B*. 3(2):1176–1177.
- 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.
- Wang Z, Wang D, Wang M, Li W, Sui Q. 2019. *Metaplexis japonica* seed hair fiber: a member of natural hollow fibers and its characterization. *Text Res J*. 89(21–22):4363–4372.
- Wei L, Yang M, Huang L, Li JL. 2019. Antibacterial and antioxidant flavonoid derivatives from the fruits of *Metaplexis japonica*. *Food Chem*. 289:308–312.
- Wei X-F, Zeng S-J, Zhang G-Q, Tang G-D, Huang J-X. 2020. Complete plastome sequence of *Hoya carnosia* (L. f.) R. Br. (Apocynaceae). *Mitochondrial DNA Part B*. 5(1):522–523.