

## Characterization of the complete chloroplast genome sequence of Chinese endemic species of *Nouelia insignis* (Hyalideae, Asteraceae) and its phylogenetic implications

Xiaofeng Liu<sup>a</sup>, Maoyun Han<sup>f</sup>, Jia Chen<sup>b</sup>, Xun Zhang<sup>c</sup>, Zhiyu Chen<sup>a</sup>, Yi Tang<sup>a</sup>, Tianmeng Qu<sup>a</sup>, Chunping Huang<sup>a,d</sup>, Shuhua Yu<sup>a,d</sup> and Zhixi Fu<sup>a,d,e</sup>

<sup>a</sup>College of Life Sciences, Sichuan Normal University, Chengdu, China; <sup>b</sup>Chengdu Foreign Languages School, Chengdu, China; <sup>c</sup>Jinjiang Experimental School, Chengdu, China; <sup>d</sup>Institute of Application and Development of Plant Resources, Sichuan Normal University, Chengdu, China; <sup>e</sup>Sustainable Development Research Center of Resources and Environment of Western Sichuan, Sichuan Normal University, Chengdu, China; <sup>f</sup>Natural Resources Bureau of Maoxian, Maoxian, China

### ABSTRACT

This study was the first report complete chloroplast genome of *Nouelia insignis* (Asteraceae, Hyalideae), the large shrubs to small trees endemic to China. The circular whole cp genome of *N. insignis* was 151,524 bp in length, containing a large single-copy (LSC) region of 83,145 bp and a small single-copy (SSC) region of 18,261 bp. These two regions were separated by a pair of inverted repeat regions (IRa and IRb), each of them 25,060 bp in length. A total of 135 functional genes were encoded, consisting of 89 protein-coding genes, 38 tRNA genes, and eight rRNA genes. The overall GC content of the chloroplast genome sequence was 37.8%, and the GC contents of the LSC, SSC, and IR regions were 35.9, 31.5, and 43.2%, respectively. The phylogenetic analysis by the Bayesian analysis showed that the species of *N. insignis* was sister group with *Gerbera jamesonii* by strong support values, and thus was closely related to members of subfamilies of Cichorioideae and Pertyoideae. These results will be useful for the future studies of Asteraceae in the worldwide.

### ARTICLE HISTORY

Received 2 December 2020  
Accepted 18 April 2021

### KEYWORDS

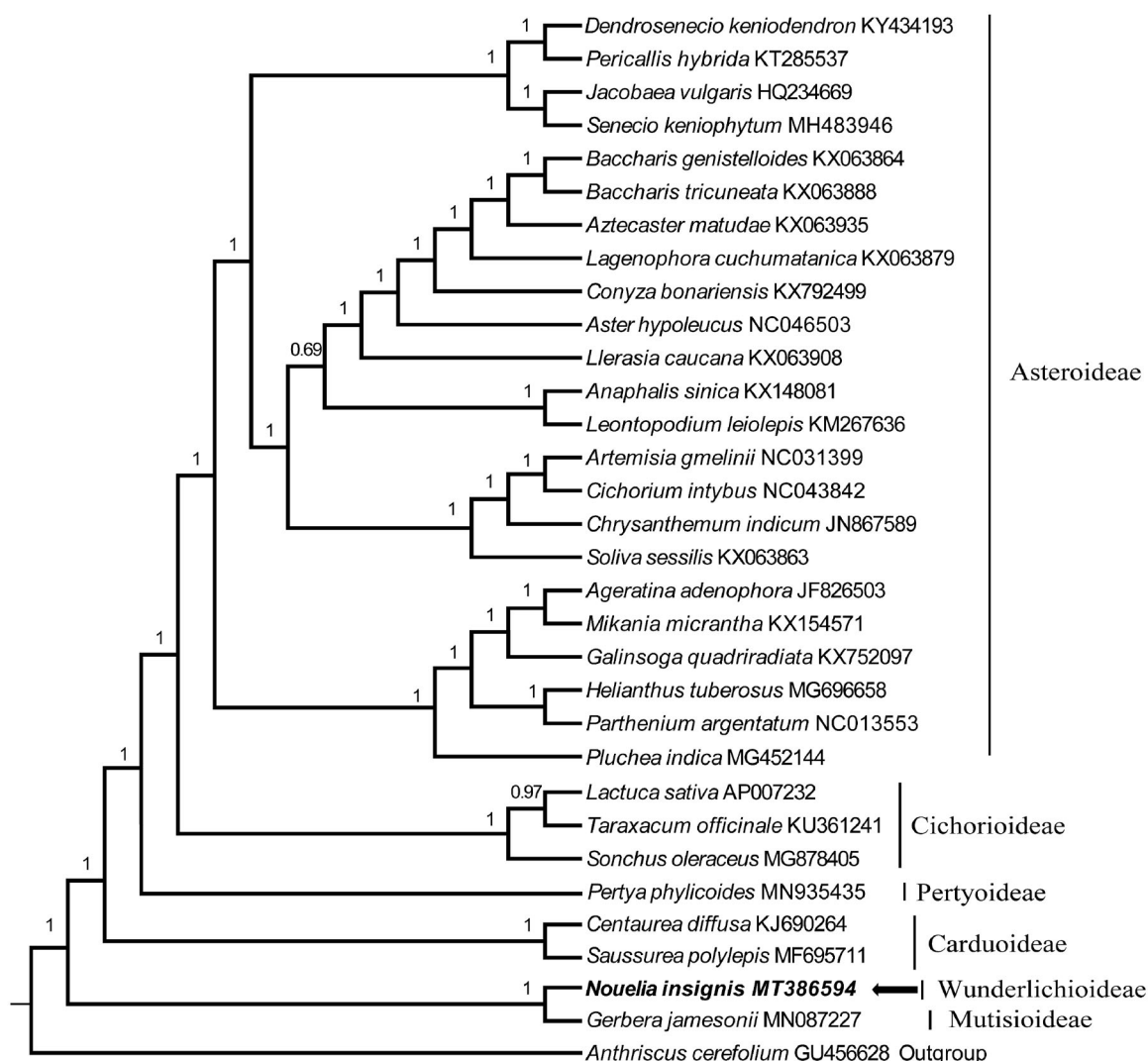
*Nouelia insignis*; complete chloroplast genome; phylogenomics analysis

The species of *Nouelia insignis* Franch. (Asteraceae, Hyalideae) is a genus endemic to southwest China (Hind 2007; Gao and Hind 2011; Gong et al. 2011; Fu et al. 2016). They are narrowly and allopatrically distributed species, separated by the important biogeographic boundary Tanaka Line in Southwest China (Zhao and Gong 2015). Genetic knowledge of *N. insignis* would provide information for protection of this wild germplasm resource. Here, we obtained the complete plastome of *N. insignis* by Illumina sequencing technology (San Diego, CA). The complete plastome reported here will contribute to the further studies on the phylogenetic analysis of *N. insignis*.

Fresh leaves of *N. insignis* were collected from Wulaxi village (101°39'36"E, 28°37'12"N), Jiulong county, Sichuan Province, China. A specimen was deposited at the botany herbarium of Sichuan Normal University, SCNU (Associate Professor, Dr. Zhixi Fu, [fuzx2017@sicnu.edu.cn](mailto:fuzx2017@sicnu.edu.cn)) under the voucher number Z.X. Fu 2862. High quality total genomic DNA was extracted from ca. 6 cm<sup>2</sup> sections of the silica-dried leaf using improved Tiangen Plant Genomic DNA Kits, add the 4 µl RNaseA and 20 µl Proteinase K after incubated (65 °C). Total DNA was directly constructed short-insert of 150 bp in length libraries and sequenced on the Illumina Genome Analyzer (HiSeq 2000) based on the manufacturer's

protocol (Illumina, San Diego, CA) by ORI-GENE (Beijing, China). Generally, more than 5.2 Gb of data was obtained for complete cp genome of *N. insignis*; *De novo* assembly of CLC Genomic Workbench v11 (CLC Bio, Aarhus, Denmark) and consensus sequence of Geneious R11.1.5 (Biomatters Ltd., Auckland, New Zealand) with referenced chloroplast genome sequence of *Gerbera jamesonii* (accession no.: MN087227). The chloroplast genome was annotated using a web-based annotation program GeSeq (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>) and editing by manual and imagining with OGDRAW v1.2 (Lohse et al. 2013).

The complete chloroplast genome of *N. insignis* was 151,524 bp in length and a typical circular structure. The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession no. MT386594. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA694507, SRP303716, and SAMN17526016 (SRS8144786), respectively. It includes a pair of inverted repeat (IR) of 25,060 bp divided by a large single-copy (LSC) region of 83,145 bp and a small single-copy (SSC) region of 18,261 bp. The general G + C content was 37.8% in the whole sequence and the corresponding values in the LSC, SSC, and IR regions are 35.9%, 31.5%, and 43.2%, respectively. The whole



**Figure 1.** The Bayesian inference (BI) phylogram inferred from 31 chloroplast genomes in Asteraceae (bootstrap value are indicated on the branches). The position of *Nouelia insignis* is in bold.

genome contained 135 genes, including 89 protein-coding genes, eight ribosomal RNA genes, and 38 tRNA genes, nevertheless, 114 unique genes, 20 genes duplicated in the IRs. In addition, among the annotated chloroplast genomic sequence, 15 genes possessed only single intron, two genes (*ycf3* and *clpP*) possessed two introns.

To identify the phylogenetic position of *N. insignis*, we used a total of 30 additional complete cp genomes of the family Asteraceae and one outgroup taxa to clarify the phylogenetic position of *N. insignis* (Figure 1). All of the cp genome sequences were aligned in MAFFT (Kato and Standley 2013). A maximum-likelihood analysis based on the GTRGAMMA model was performed with Bayesian method on the CIPRES (Miller et al. 2010; Ronquist et al. 2012) using 1000 bootstrap replicates. The phylogenetic analysis of the cp genome dataset recovers the similar clades as in previous phylogenetic work (Panero and Funk 2008; Panero et al. 2014; Fu et al. 2016). The Bayesian inference (BI) result with 100% bootstrap showed that *N. insignis* has a close sister relationship with the genus *Gerbera* (Figure 1). The complete

cp genome sequence of *N. insignis* will be the valuable resource for future studies on taxonomy and phylogeny of family Asteraceae and provides useful molecular data for further phylogenetic and evolutionary analysis.

## Disclosure statement

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

## Funding

This work was supported by the National Natural Science Foundation of China under Grant [32000158], The Special Research Project of National Tradition Chinese Medicine Industry, the Fourth National Survey on Chinese Material Medica Resources under Grant [GZY-KJS-2018-004]; The Sichuan Science and Technology Research Projects of Traditional Chinese Medicine under Grant [2018PC005]; the Project of Sustainable Development Research Center of Resources and Environment of Western Sichuan under Grant [2020CXZYHJZX03]; the Philosophy and Social Science Key Research Base Project of Sichuan Province, Sichuan Nationalities and Mountain Economy Development Research Center

under Grant [SDJJ1907]; The Research and Demonstration of Fast Restoration Model of Forest and Grass Vegetation Program in the Damaged Habitat of Rare Animals of Jiuzhaigou Earthquake Stricken Area [2018-128].

### Data availability statement

The data that newly obtained at this study are openly available in the NCBI (<https://www.ncbi.nlm.nih.gov/>) under accession number of MT386594. Raw sequencing reads were deposited in SRA with BioProject accession (PRJNA694507) (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA694507>).

### References

- Fu ZX, Jiao BH, Nie B, Zhang GJ, Gao TG. 2016. A comprehensive generic-level phylogeny of the sunflower family: implications for the systematics of Chinese Asteraceae. *J Syst Evol.* 54(4):416–437.
- Gao TG, Hind DJN. 2011. *Nouelia*. In: Wu CY, Raven PH, Hong DY, editors. *Flora of China*. Vols. 20–21. Beijing/St. Louis: Science Press/Missouri Botanical Garden Press; p. 10.
- Gong X, Luan SS, Hung KH, Hwang CC, Lin CJ, Chiang YC, Chiang TY. 2011. Population structure of *Nouelia insignis* (Asteraceae), an endangered species in southwestern China, based on chloroplast DNA sequences: recent demographic shrinking. *J Plant Res.* 124(2):221–230.
- Hind DJN. 2007. *Nouelia*. In: Kadereit J, Jeffrey C, editors. *The families and genera of vascular plants*. Vol. 8. Berlin and Heidelberg: Springer; p. 119.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 30(4):772–780.
- Lohse M, Drechsel O, Kahlau S, Bock R. 2013. Organellar Genome DRAW—a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. *Nucleic Acids Res.* 41:575–581.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*; New Orleans; p. 1–8.
- Panero JL, Funk VA. 2008. The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. *Mol Phylogenet Evol.* 47(2):757–782.
- Panero JL, Susana EF, Espinar LA, Crozier BS, Barboza GE, Cantero JJ. 2014. Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae. *Mol Phylogenet Evol.* 80:43–53.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 61(3):539–542.
- Zhao YJ, Gong X. 2015. Genetic divergence and phylogeographic history of two closely related species (*Leucomeris decora* and *Nouelia insignis*) across the ‘Tanaka Line’ in Southwest China. *BMC Evol Biol.* 15:134.