

## The complete chloroplast genome of *Torenia violacea* (Azaola) Pennell 1943 (Linderniaceae)

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### ABSTRACT

*Torenia violacea* (Azaola) Pennell 1943 as a traditional Chinese medicine plant, has been used to treat multiple diseases. In this study, we sequenced, assembled, and characterized the complete plastome of *T. violacea*. The plastome (OQ167784) exhibited a typical quadripartite structure, with a total length of 154,007 bp, comprising a pair of inverted repeats (IRs; 24,809 bp) separated by a large single-copy (LSC) region (85,559 bp) and a small single-copy (SSC) region (18,830 bp). Through genome annotation, we identified 133 genes, including 87 protein-coding genes, eight rRNA genes, and 38 tRNA genes. Phylogenetic analysis revealed a close relationship between *T. violacea* and *T. fournieri*. The research results provide basic genetic resources for the development of species identification and investigation of phylogenetic relationships in the *Torenia* genus.

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### KEYWORDS

*Torenia violacea*; complete chloroplast genome; *Torenia* genus; phylogenetic analysis



### Introduction


*Torenia violacea* (Azaola) Pennell 1943, is an annual herbaceous plant belonging to the *Torenia* genus of the Linderniaceae family, and widely distributed in China. It has a

high ornamental value due to its showy purple-red flowers (Figure 1). The whole plant was also used as a traditional Chinese medicinal herb for clearing heat, expelling dampness, relieving coughs, and other pharmacological effects.



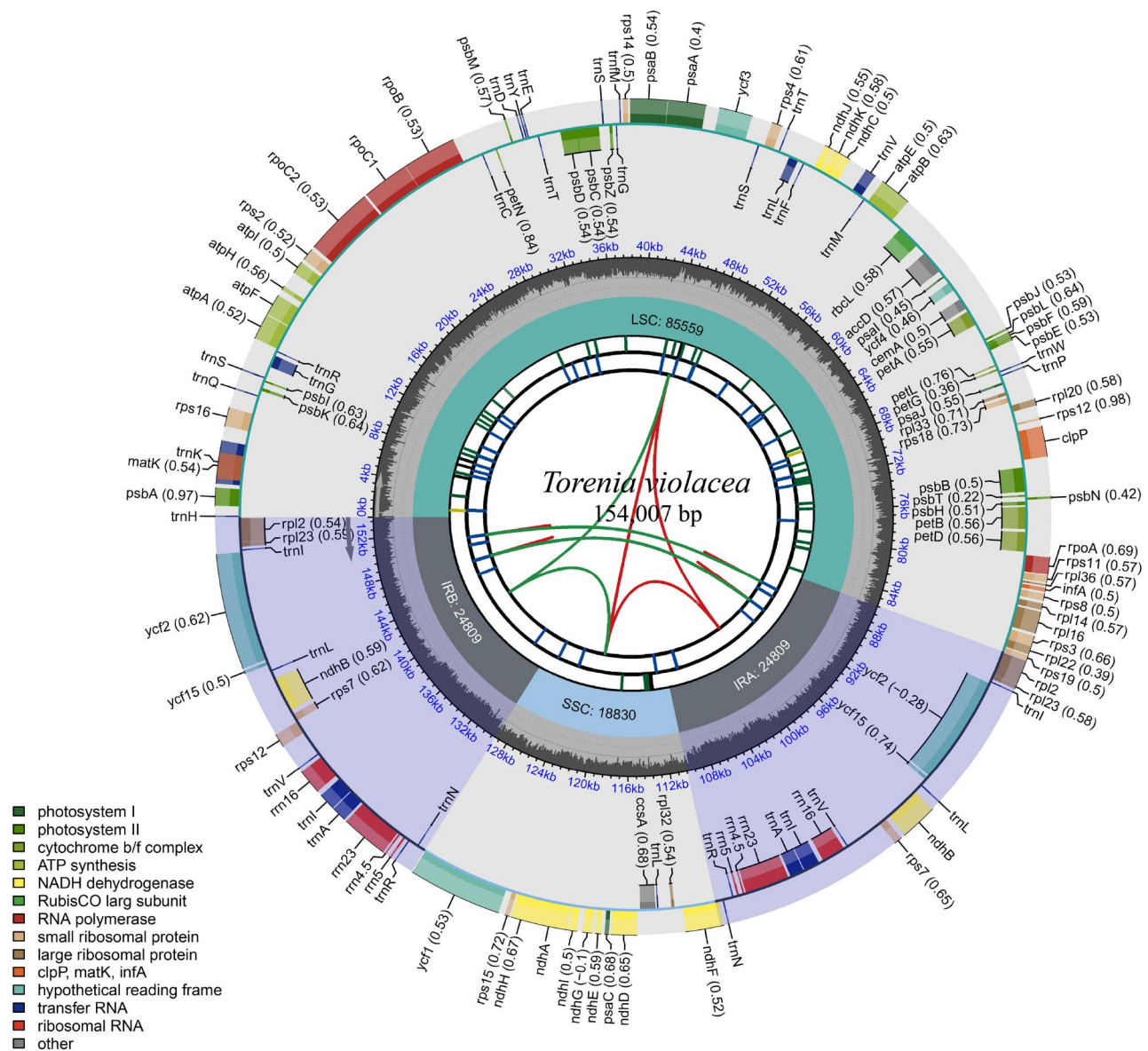
**Figure 1.** Photograph of *Torenia violacea* (the photograph was taken by prof. Xiaoyan Xiang in Mt. Huangshan, China). The foliage of *T. violacea* exhibits ovate or narrowly ovate shapes, characterized by villous, shallowly serrate margin, acuminate apex. The flowers are in terminal fascicles or solitary in leaf axils, rarely in racemes. Furthermore, the calyx of *T. violacea* displays purple-red and the corollas pale yellow or white.

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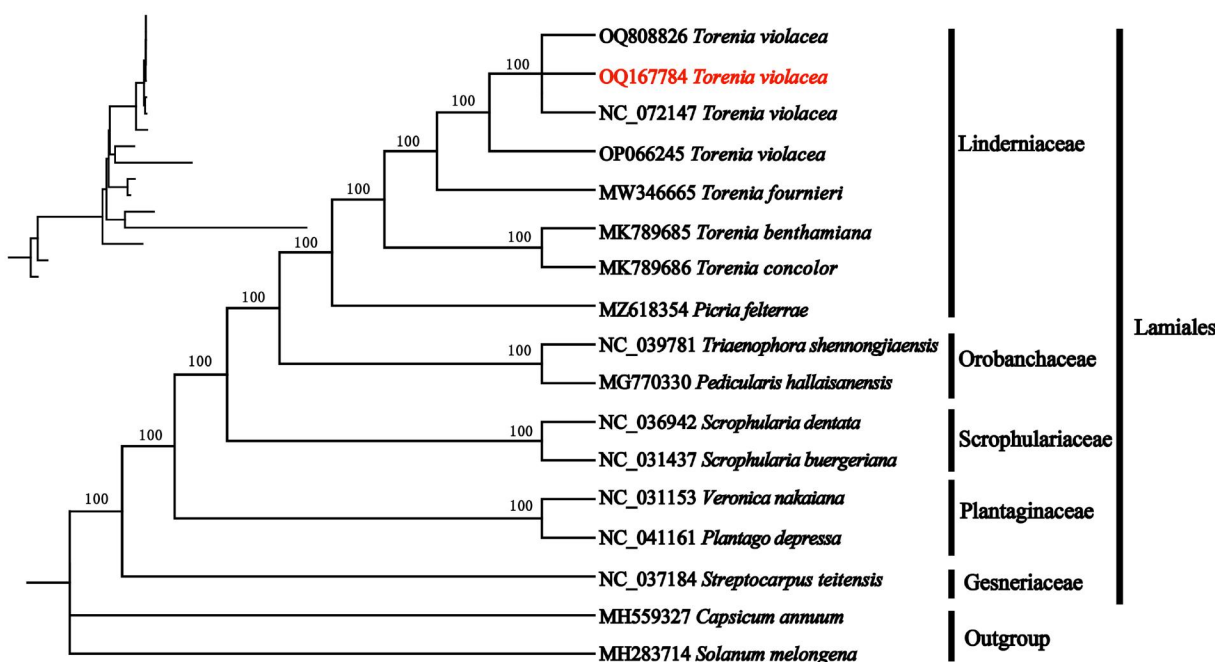
**Figure 2.** Schematic map of overall features of the chloroplast genome of *Torenia violacea*. The boxes of different sizes and colors on the outermost circle represent genes and their lengths. The inner and outer boxes of the outermost circle represent genes transcribed clockwise and counter-clockwise. The gray area in the middle circle represents the changes in GC content at different positions, and the regions and lengths represented by the tetrad structure (LSC, SSC, IRA, and IRb) are drawn in different colors on the inner circle.

Morphologically, *T. violacea* is very similar to other species of the genus *Torenia* and can only be distinguished by the detailed characteristics of the flowers (China 2013). Before flowering, morphology of the leaves is insufficient to distinguish these species. Therefore, molecular data are required for species delimitation. With the rapid development of high-throughput sequencing technology, the whole chloroplast genome sequence has been widely used for phylogenetic and species delimitation studies (Li et al. 2022). To date, there have not been any laboratories using the whole plastome of *T. violacea* for research for molecular biology species identification. Consequently, we employed next-generation sequencing technology and phylogenetic analysis in this study to publish the whole chloroplast genome of *T. violacea* and enhance the species delimitation in *Torenia*.

## Materials and methods

Fresh leaves of *T. violacea* were collected from Tangkou, Huangshan, Anhui Province, China (30.024°N, 118.188°E) and dried with silica gel. The voucher specimens were deposited in the Herbarium of Anqing Normal University (voucher number: ZLP092302; contact person: Xiaoyan Xiang; email: xiaoyanxiang@aqnu.edu.cn). Total DNA was extracted from dried leaves using the plant genomic DNA kit (Tiangen Biochemical Technology Co., Ltd., Beijing, China).

The DNA library construction and 150-bp paired-end sequencing were performed using the Illumina NovaSeq 6000 platform (Novogene, Tianjin, China), yielding approximately 2 GB of data. Checking and improving the integrity and quality of the whole plastome sequences with *Torenia fordii* (NCBI accession number: MW309811) as reference



**Figure 3.** Phylogenetic relationships of *Torenia violacea* and other species of Lamiales inferred from ML analyses based on the whole chloroplast genome, with *Solanum macrocarpon* and *Capsicum annuum* as the outgroup. The numbers on each branches indicate the boot support value of the ML analyses. The sequences used for tree construction are as follows: *T. violacea* (OQ808826, N\_C\_072147, OP066245), *T. fournieri* (MW346665), *T. concolor* (MK789685, Cheng, Li, et al. 2019), *T. benthamiana* (MK789686, Cheng, Liu, et al. 2019), *Picria felterrae* (MZ618354), *Pedicularis hallaisanensis* (MG770330, Cho et al. 2018), *Triaenophora shennongjiaensis* (NC\_039781, Xia and Wen 2018), *Scrophularia buergeriana* (NC\_031437, Yi and Kim 2016), *Scrophularia dentata* (NC\_036942), *Veronica nakaiana* (NC\_031437, Choi et al. 2016), *Plantago depressa* (NC\_041161), *Streptocarpus teitensis* (NC\_037184, Zhao et al. 2019), *C. annuum* (MH559327, D'Agostino et al. 2018), and *S. macrocarpon* (MH283714, Aubriot et al. 2018).

plastome, through reference guided mapping using BWA (v0.7.17), SAMtools (v1.13), and IGV (2.15.2), obtained a more accurate plastome genome. The minimum and average read mapping depths for assembled genomes were  $\times 584$  and  $\times 4704$  (Figure S1), respectively.

The chloroplast genome was assembled using the GetOrganelle pipeline (Jin et al. 2020) and annotation was conducted with Plastid Genome Annotator (Qu et al. 2019). The annotated chloroplast genome sequence of *T. violacea* has been deposited in GenBank of the National Center for Biotechnology Information (NCBI accession number: OQ167784). The plastome map of *T. violacea* was drawn through CPGview (Liu et al. 2023). The phylogenetic relationship of *T. violacea* was reconstructed using the maximum-likelihood (ML) method based on the multiple alignment of *Torenia* species and seven previously reported chloroplast genomes of Lamiales, with *Capsicum annuum* (MH559327) and *Solanum macrocarpon* (MH283714) as the outgroup. All sequences were aligned with MAFFT v.7 (Katoh and Standley 2013) using the default settings. ML analysis was conducted using RAXML-HPC v.8.2.8 (Stamatakis 2014) with 1000 bootstrap replicates on the CIPRES Science Gateway (<https://www.phylo.org/>), and the (GTR) + G + I model being used in ML analyses.

## Results

The plastome of *T. violacea* was 154,007 bp and shared the common quadripartite structure of most angiosperms (Figure 2). The genome comprises two copies of inverted repeat (IR) (24,809 bp each) separated by a large single-copy (LSC)

region (85,559 bp) and a small single-copy (SSC) region (18,830 bp). A total of 133 genes were identified in the cp genome of *T. violacea*, containing 87 protein-coding genes (PCGs), eight ribosomal RNA genes (rRNAs), and 38 transfer RNA genes (tRNAs). Of these, 11 genes (*rsp16*, *atpF*, *rpoC1*, *ycf3*, *clpP*, *petB*, *petD*, *rpl16*, *rp12*, *ndhB*, and *ndhA*) are cis-spliced, and *rps12* is also a trans-spliced gene (Figure S2). The results of phylogenetic research based on ML indicate that *T. violacea* belonged to the genus *Torenia* in the Linderniaceae family. The *T. violacea* and *T. fournieri* were sister to each other (Figure 3).

## Discussion and conclusions

In this study, the complete plastome of *T. violacea* was sequenced for the first time, exhibiting a total length of 154,007 bp. A total of 133 genes were annotated, comprising 87 PCGs, eight rRNA genes, and 38 tRNA genes. There were no significant differences in genome size and gene content between *T. violacea* and other chloroplast genomes in *Torenia* (Cheng, Li, et al. 2019; Cheng, Liu, et al. 2019). Phylogenetic analysis indicates a sister relationship between *T. violacea* and *T. fournieri*. This suggests a close genetic relationship between the two *Torenia* genus plants. In addition, species from different families cluster into different monophyletic groups with a 100% bootstrap value. This indicates that chloroplast genomic data can construct robust phylogenetic relationships. In conclusion, our research results provide basic genetic resources for the development of species identification and the investigation of phylogenetic relationships in the *Torenia* genus.

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## Ethical approval

The sampling site is not located in any protected area. The research was conducted with the permission of Anqing Normal University of China.

## Author contributions

X.Y. Xiang planned and designed the experiments, and drafted the paper; W.H. Zhu and H.Q. Cui performed the experiments and analyzed the data; all authors revised the final version of the paper and have reviewed and approved the manuscript.

## Disclosure statement

The authors report there are no competing interests to declare.

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## Data availability statement

The genome sequence data supporting the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under accession no. OQ167784. The associated BioProject, SequenceRead Archive, and BioSample numbers are PRJNA918330, SRR22967894, and SAMN32573999.

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