

## The complete chloroplast genome sequence of *Athrotaxis cupressoides*

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### ABSTRACT

*Athrotaxis cupressoides* (Cupressaceae) is a Tasmanian endemic conifer. It is mainly threatened by habitat fragmentation and limited distribution, yet little is known about its genomic background. In this study, the complete chloroplast (cp) genome of the species was assembled and annotated. The cp genome of *A. cupressoides* is a circular of 134,248 bp in size, where the inverted repeat regions (IRs) were lost. A total of 112 unique genes were annotated, including 78 proteins-coding genes, four *rRNA* genes, and 30 *tRNA* genes. The maximum likelihood (ML) phylogenetic tree indicates that the subfamily Athrotaxidoideae, to which *A. cupressoides* belongs, is sister to a clade including four subfamilies including Sequoioideae, Taxodioideae, Cupressioideae, and Callitroideae. The complete cp genome will be helpful to further studies on the conservation of this species and the evolutionary history of Cupressaceae.

### ARTICLE HISTORY

Received 15 August 2021  
Accepted 31 October 2021

### KEYWORDS

Chloroplast genome;  
*Athrotaxis cupressoides*;  
phylogenetic tree

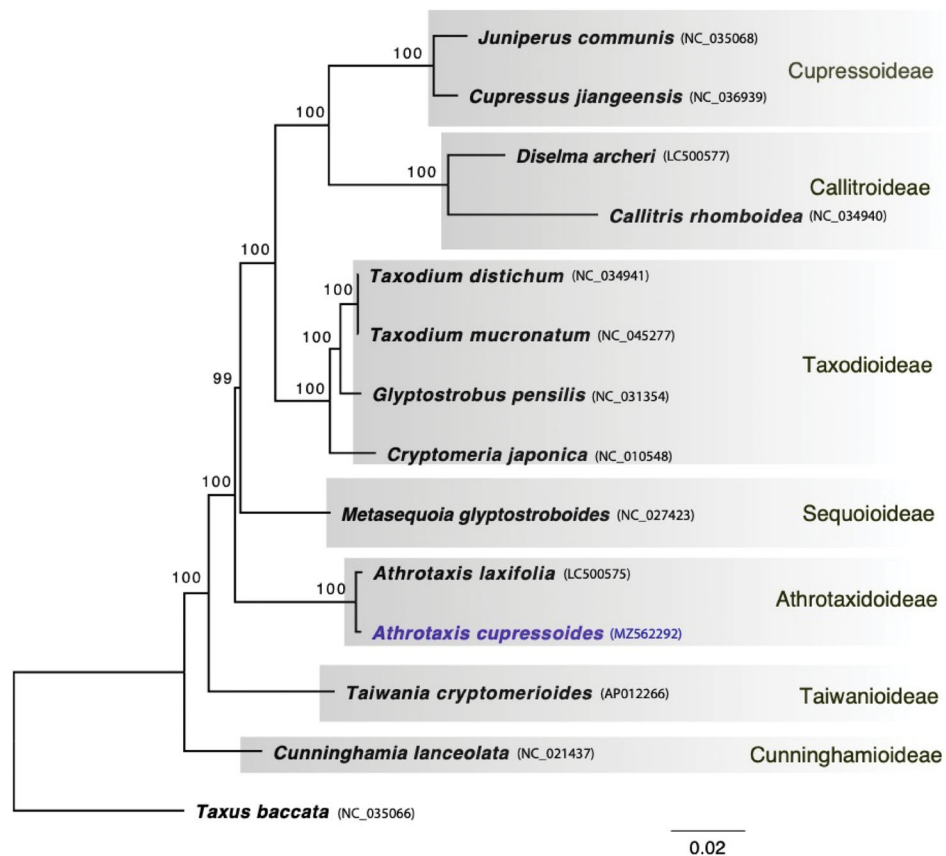
*Athrotaxis cupressoides*, which belongs to Cupressaceae, is an endemic species of Tasmania, Australia. It mainly distributes in montane to subalpine habitats (700–1300 m .a.s.l.) on the Central Plateau, the Great Western Tier, and westward mountains in Tasmania (Farjon 2019). In 2012, IUCN Red List assessed *A. cupressoides* as Vulnerable due to its small extent of occurrence (EOO) and area of occupancy (AOO), and severely fragmented habitat (Farjon 2013). Previous studies on *A. cupressoides* focused on ecological differentiation, conservation, climate change, and fire ecologies (Cullen and Kirkpatrick 1988; Kirkpatrick 1989; Enright and Hill 1995; Balmer et al. 2003; Pyrke and Marsden-Smedley 2005; Kirkpatrick et al. 2010), yet little is known about its genomic background. In this study, we report the complete chloroplast (cp) genome of *A. cupressoides* and infer a phylogenomics tree that comprising this species and other representative species of Cupressaceae.

The material was sampled from a voucher specimen in the herbarium of Royal Botanic Garden Edinburgh (RBGE; collector: Gillanders, Kenneth; Barcode: E00420831), which was collected from Tasmania, Australia (43°13'20.1''S 146°22'04.5''E). Total genomic DNA was extracted from dried leaves sample using a modified CTAB protocol (Doyle 1991). Whole genome re-sequencing was conducted on the Illumina HiSeq platform (Illumina, San Diego, CA), where a library with insertion size of 500 bp was constructed and sequenced with paired-end (150 bp) reads. A total of 2038 Mb clean reads were used for de novo assembly with GetOrganelle version 1.7.5 (Jin et al. 2020). Gene annotation was performed via Plann version 1.1.2 (Huang and Cronk 2015).

The complete cp genome of *A. cupressoides* (GenBank accession number MZ562292) was a circular molecule of 134,248 bp in length, where the inverted repeat regions (IRs) were lost. The overall GC content was 34.25%. A total of 110 genes were encoded, of which 112 were unique and two were duplicated. Among the unique genes, 78 were protein-coding genes, four were *rRNA* genes and 30 were *tRNA* genes.

Phylogenetic analysis was performed based on complete cp genome of *A. cupressoides* and 12 species that represent the other six subfamilies of Cupressaceae. *Taxus baccata* (Taxaceae) was selected as the outgroup. The genome-wide alignment of 14 genomes was performed with progressive Mauve (Darling et al. 2010) and MAFFT version 7.487 (Katoh and Standley 2013), and the poorly aligned regions were filtered by Divvier (Ali et al. 2019). The maximum-likelihood (ML) method was performed in RAxML version 8.2.12 (Stamatakis 2014) using the GTRGAMMA model. Node support was assessed by 1000 rapid bootstrap replicates.

According to the ML tree, *A. cupressoides* was sister to *A. laxifolia* with a high support (bootstrap support value = 100; Figure 1), and the Athrotaxidoideae was basal to all other subfamily of Cupressaceae except for the Taiwanioidae and Cunninghamioideae, which is consistent with previous research on Cupressaceae phylogeny (Mao et al. 2012). However, we found a short internode distance between the most recent common ancestor (MRCA) of Athrotaxidoideae and Cupressioideae, and the MRCA of Sequoioideae and Cupressioideae (Figure 1), despite high bootstrap support for both nodes. Our findings could provide helpfully genomic



**Figure 1.** Maximum likelihood (ML) phylogenetic tree based on chloroplast genomes of 14 species was constructed using RAxML version 8.2.12. The bootstrap support values are shown by numbers for each node. The analyzed species and corresponding Genbank accession numbers are shown in the tree.

resources for future studies on the conservation of this species and the evolutionary history of Cupressaceae.

## Acknowledgments

The authors thank Royal Botanic Garden Edinburgh for providing dried leaf sample of *A. cupressoides*.

## Disclosure statement

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

## Funding

This work is financially supported by National Natural Science Foundation of China [grant No. U20A2080; 31622015] and Sichuan University [Fundamental Research Funds for Central University Basic Research Fund of China, SCU2020D003, SCU2021D006].

## Data availability statement

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. MZ562292. The associated BioProject, SRA, and bio-sample numbers are PRJNA772053, SRR16379974, and SAMN22358055, respectively.

## References

- Ali RH, Bogusz M, Whelan S. 2019. Identifying clusters of high confidence homologies in multiple sequence alignments. *Mol Biol Evol.* 36(10): 2340–2351.
- Balmer J, Whinam J, Kelman J, Kirkpatrick JB, Lazarus E. 2003. Floristic values of the Tasmanian wilderness world heritage area. Nature Conservation Branch, Department of Primary Industries, Water and Environment, Tasmania, Australia.
- Cullen PJ, Kirkpatrick JB. 1988. The ecology of *Athrotaxis* D. Don (Taxodiaceae). 2. The distribution and ecological differentiation of *Athrotaxis cupressoides* and *Athrotaxis selaginoides*. *Austral J Ecol.* 36(5):561–573.
- Darling AE, Mau B, Perna NT. 2010. Progressive Mauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One.* 5(6): e11147.
- Doyle JJ. 1991. DNA protocols for plants-CTAB total DNA isolation. In: Hewitt GM, Johnston A, editors. *Molecular techniques in taxonomy*. Berlin, Germany: Springer; p. 283–293.
- Enright NJ, Hill RS. 1995. *Ecology of the southern conifers*. Carlton, Australia: Melbourne University Press.
- Farjon A. 2013. *Athrotaxis cupressoides*. The IUCN red list of threatened species. 2013e.T32054A2810019. <https://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T32054A2810019.en>. Downloaded on 03 August 2021.
- Farjon A. 2019. *Athrotaxis cupressoides*, from the website: 'threatened conifers of the world.' <https://threatenedconifers.rbge.org.uk/conifers/athrotaxis-cupressoides>. Downloaded on 3 August 2021.
- Huang DI, Cronk QC. 2015. Plann: a command-line application for annotating plastome sequences. *Appl Plant Sci.* 3(8):1500026.
- Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol.* 21(1):241.

- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 30(4):772–780.
- Kirkpatrick J. 1989. The conservation and reservation status of Tasmanian higher plants. Hobart, Australia: Department of Parks, Wildlife and Heritage.
- Kirkpatrick JB, Bridle K, Dickinson JM. 2010. Decades-scale vegetation change in burned and unburned alpine coniferous heath. *Aust J Bot.* 58(6):453–462.
- Mao K, Milne RI, Zhang L, Peng Y, Liu J, Thomas P, Mill RR, Renner SS. 2012. Distribution of living Cupressaceae reflects the breakup of Pangea. *Proc Natl Acad Sci.* 109(20):7793–7798.
- Pyrke AF, Marsden-Smedley JB. 2005. Fire-attributes categories, fire sensitivity, and flammability of Tasmanian vegetation communities. *Tasforests.* 16:35–46.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 30(9):1312–1313.