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TECHNICAL NOTE

Characterization of the whole chloroplast genome of a rare and endangered species *Aconitum reclinatum* (Ranunculaceae) in the United States

Hanghui Kong[1,4] · Wanzhen Liu[2] · Gang Yao[3] · Wei Gong[2]

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Abstract The complete chloroplast genome (cp genome) of *Aconitum reclinatum* (Ranunculaceae) was characterized through Illumina paired-end sequencing. The cp genome is circle and 157,354 bp in length, consisting of a pair of 26,061 bp inverted repeat regions (IRs) which are separated by a large single copy region (LSC) of 88,269 bp and a small single copy region (SSC) of 16,963 bp. The cp genome contains 135 genes, including 87 protein-coding genes (PCGs), 40 tRNA genes (tRNA) and eight ribosomal RNA genes (rRNA). Among these, seven PCGs, eight tRNA and four rRNA are duplicated. The overall GC content of the *A. reclinatum* cp genome is 38.00%, while the corresponding values of the LSC, SSC and IRs regions are 36.00, 32.80 and 43.00%, respectively. The phylogenetic analysis suggested that *A. reclinatum* is closely related to *A. barbatum* var. *hispidum and A. barbatum* var. *puberulum* with high bootstrap support of 100%.

Keywords *Aconitum reclinatum* · Chloroplast genome · Endangered species · Illumina sequencing · Phylogenetic analysis

✉ Wei Gong
wgong@scau.edu.cn

[1] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[2] College of Life Sciences, South China Agricultural University, Guangzhou 510614, China

[3] College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510614, China

[4] Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Aconitum reclinatum A. Gray, known as trailing white monkshood, belongs to the genus of *Aconitum* in the family of Ranunculaceae. It is an herbaceous perennial plant with greenish white flowers. This species is listed as a national wetland plant, which is rare, threatened and endemic to the states of North Carolina, Tennessee, Virginia, West Virginia and Pennsylvania in the United States (Kintsch and Urban 2002; Lichvar 2013). Aconite produced from the roots of *Aconitum* is widely used for different diseases (Xiao et al. 2005). Many species in the genus *Aconitum* are proved to be essential components in the formulations of traditional herbal medicine in Asia. Even though some species are highly toxic because of aconite alkaloid, they possess a variety of medicinal importance (Semenov et al. 2016; Liang et al. 2016). *Aconitum reclinatum* is the only species of *Aconitum* subg. *Lycoctonum* in the New World (Tamura and Lauener 1979). An improved understanding of *A. reclinatum* genomic information would contribute to formulate the comprehensive conservation strategy to protect and restore this species. However, genomic level of diversity is still unclear for this species. In the current study, we presented the complete chloroplast genome of *A. reclinatum* (GenBank Accession Number: MF155665) based on the Illumina sequencing platform.

Total genomic DNA was extracted from the fresh leaves of a single individual of *A. reclinatum* with the modified CTAB method (Doyle and Doyle 1987). The DNA concentration was quantified using a Nanodrop spectrophotometer (Thermo Scientific, Carlsbad, CA, USA). The final DNA concentration >30 ng/μL were chosen for further Illumina sequencing. The sequences of the cp genome of *A. reclinatum* were amplified using fifteen universal primer pairs developed by Zhang et al. (2016). A paired-end library was constructed by Nextera XT DNA Library Prep Kit (Illumina Inc., San Diego, CA, USA), and then 250 bp paired

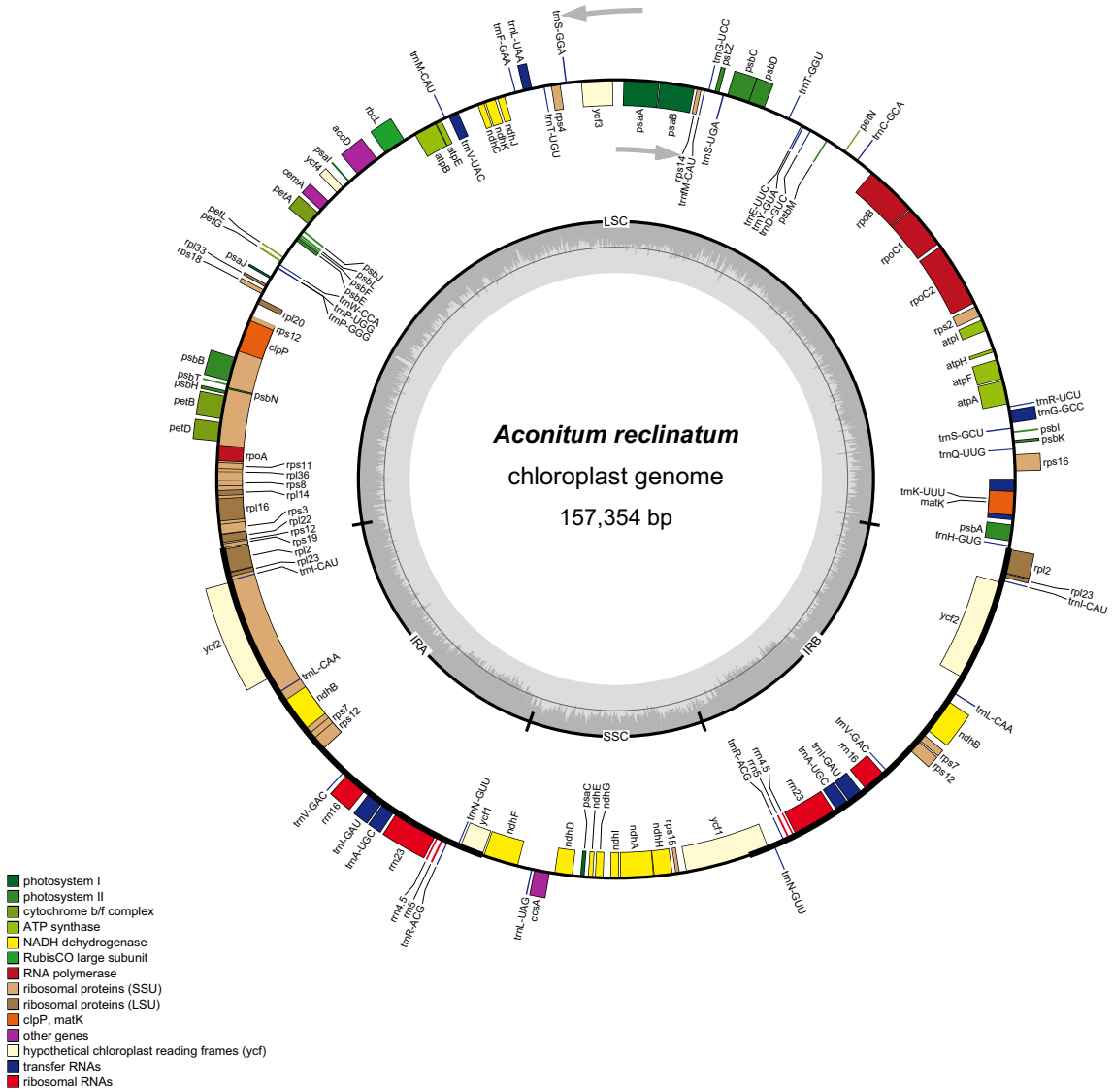

Fig. 1 Gene map of the *Aconitum reclinatum* chloroplast genome

reads were sequenced using the Illumina Miseq Desktop Sequencer. Reads of the *A. reclinatum* cp genome were initially assembled using CLC Genomics Workbench v7.5.1 (CLC Bio., Aarhus, Denmark) with 60 and 64 k-mer respectively. The contigs were checked against the reference cp genome of *A. barbatum* var. *hispidum* (KT820664) and *A. barbatum* var. *puberulum* (KC844054) (Chen et al. 2015). A complete *A. reclinatum* cp genome sequence was subsequently constructed by manual assembling of the hitting contigs. The resulting cp genome sequence was used as a reference, which was subsequently verified by remapping initial reads using GENEIOUS R9.1.4 (Biomatters Ltd., Auckland, New Zealand). The annotation of the *A. reclinatum* cp genome sequence was performed using DOGMA (http://dogma.ccbb.utexas.edu/) (Wyman et al. 2004) and also conducted on the program GENEIOUS R9.1.4 (Biomatters Ltd., Auckland, New Zealand) by comparing with the cp genome of *A. barbatum* var. *hispidum* and *A. barbatum* var. *puberulum*. The annotations of tRNA genes were further confirmed using ARAGORN (Laslett and Canback 2004) followed by manual adjustment. The annotated cp genome sequence of *A. reclinatum* has been submitted to GenBank. Finally, the circular genome map was generated with OGDRAW (http://ogdraw.mpimp-golm.mpg.de/) (Lohse et al. 2013).

The cp genome of *A. reclinatum* (Ranunculaceae) is circle with 157,354 bp in length, displaying a typical quadripartite structure and consisting of a pair of inverted repeat regions (IRs) of 26,061 bp, a large single-copy region (LSC) of 88,269 bp and a small single-copy

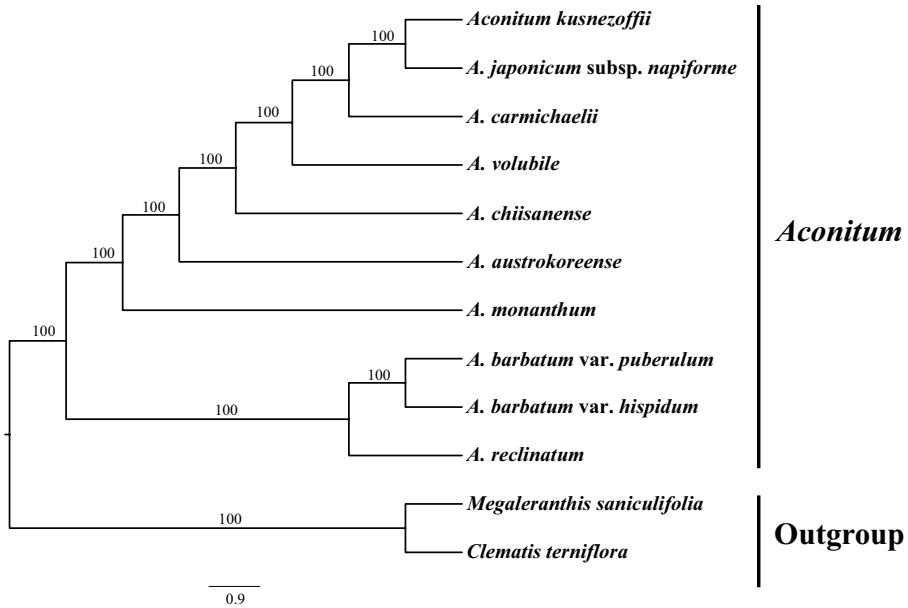

Fig. 2 Phylogenetic reconstruction of eight species and two varieties within *Aconitum* based on the concatenated sequences of 84 to 87 chloroplast PCGs using Maximum Likelihood (ML) analysis. Bootstraps with 1000 replicates were assessed and displayed on the *above of the branches*

region (SSC) of 16,963 bp (Fig. 1). It contains 135 genes, including 87 protein-coding genes (PCGs), 40 transfer RNA genes (tRNA) and eight ribosomal RNA genes (rRNA). Among these, seven PCGs (*ndhB, rpl2, rpl23, rps7, rps12, ycf2* and *ycf15*), eight tRNA (*trnA-UGC, trnI-CAU, trnI-GAU, trnL-CAA, trnN-GUU, trnR-ACG* and *trnV-GAC*) and four rRNA (*rrn4.5, rrn5, rrn16, rrn23*) were revealed to occur in double copies. The overall GC content of *A. reclinatum* cp genome is 38.00%, while the corresponding GC content of the LSC, SSC and IRs regions are 36.00, 32.80 and 43.00%, respectively.

Phylogenetic analysis was conducted based on eight species and two varieties in the genus of *Aconitum*, with *Megaleranthis saniculifolia* and *Clematis terniflora* as outgroup. The cp genome sequences from the finalized data set were aligned with MAFFT v7.0.0 (Katoh and Standley 2013). Altogether 84–87 chloroplast PCGs were used for the phylogenetic reconstruction. Maximum Likelihood (ML) analyses used the identical partition scheme and were conducted using RAxML (Stamatakis 2006) and the RAxML graphical interface [raxmlGUI v.1.3 (Silvestro and Michalak 2012)]. Phylogenetic analysis indicated that all the representative ten species from the genus *Aconitum* formed monophyly, among which *A. reclinatum* clustered with *A. barbatum* var. *hispidum* and *A. barbatum* var. *puberulum* with 100% bootstrap support value (Fig. 2).

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

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