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The complete chloroplast genome of common walnut (*Juglans regia*)

Yiheng Hu^a, Keith E. Woeste^b, Meng Dang^a, Tao Zhou^a, Xiaojia Feng^a, Guifang Zhao^a, Zhanlin Liu^a, Zhonghu Li^a and Peng Zhao^a

^aKey Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Sciences, Northwest University, Xi'an, China; ^bUSDA Forest Service Hardwood Tree Improvement and Regeneration Center (HTIRC), Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA

ABSTRACT

Common walnut (*Juglans regia* L.) is cultivated in temperate regions worldwide for its wood and nuts. The complete chloroplast genome of *J. regia* was sequenced using the Illumina MiSeq platform. This is the first complete chloroplast sequence for the Juglandaceae, a family that includes numerous species of economic importance. The chloroplast genome of *J. regia* was 160 367 bp in length, with 36.11% GC content. It contains a pair of inverted repeats (26 035 bp) which were separated by a large single copy (89 872 bp) and a small single copy region (18 425 bp). A total of 137 genes were annotated, which included 86 protein-coding genes, three pseudogenes (two *ycf15* and one *infA*), 40 tRNA genes and eight rRNA genes. The neighbour-joining phylogenetic analysis with the reported chloroplast genomes showed that common walnut chloroplasts are most closely related to those of the Fagaceae family.

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Juglans L. is one of the eight living genera in the family Juglandaceae consisting of 21 extant taxa (Manning 1978; Aradhya et al. 2007). The common walnut (*Juglans regia* L.) is the most economically important member of the genus *Juglans* because of its high-quality timber and nutritious nuts (Pollegioni et al. 2015). It is native to the mountainous regions of central Asia, but it has become the most widespread tree nut cultivated in the world (Chen et al. 2014).

Chloroplasts play an important role in photosynthesis in green plants and participate in the biosynthesis of starch, fatty acids and amino acids (Neuhaus & Emes 2000). The DNA sequence of the chloroplast genome can be used as a super barcode or a resource for research in phylogeography, genetic diversity and evolution. Although many members of the Juglandaceae are economically important, no member of the family has had its complete chloroplast sequence published.

For this study, we collected the fresh, healthy leaves from *J. regia* growing in the orchard of Northwest University, Shaanxi, China. DNA was extracted using methods described by Zhao and Woeste (2011). We sequenced the complete chloroplast genome of *J. regia* with the Illumina MiSeq sequencing platform (Sangon Biotech, Shanghai, China). We assembled the chloroplast genome using SPAdes (<http://bioinf.spbau.ru/spades>) and annotated with software CpGAVAS (<http://www.biomedcentral.com/1471-2164/13/715>) (Liu et al. 2012). We determined the complete chloroplast genome sequence of *J. regia* was 160 367 bp in length (GenBank accession number KT963008). The GC content was 36.11%. Chloroplasts are typically AT-rich, and the GC content of the *J. regia* chloroplast was similar to values previously reported for most other vascular species (e.g., 36.9% in *Quercus aliena*

Blume; Lu et al. 2015). The large single copy (LSC) and small single copy (SSC) contained 89 872 and 18 425 bp, respectively, while the inverted repeat (IR) was 26 035 bp in length. This chloroplast genome contained 137 functional genes, including 86 protein-coding genes, three pseudogenes (two *ycf15* and one *infA*), 40 tRNA genes and eight rRNA genes. There were 12 protein-coding genes, 14 tRNA and all eight rRNA genes duplicated in the IR regions. The LSC region contained 62 protein-coding and 25 tRNA genes, whereas the SSC region contained 12 protein-coding and one tRNA gene. Fourteen genes contained one or two introns, including the protein-coding genes, *rps16*, *atpF*, *rpoC1*, *ycf3* (three introns), *clpP*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB* and *rps12*.

The phylogenetic relationship of *J. regia* to other plant families was evaluated by comparing the *J. regia* chloroplast to chloroplasts from 11 other species (eight species in the Fagales), downloaded from NCBI). A neighbour-joining analysis was performed using the software Geneious version 8.0.2 (<http://www.geneious.com/>) (Figure 1). The phylogenetic tree showed that *J. regia* (Juglandaceae) was most closely related to members of Fagaceae family and the genus *Populus* (Figure 1). The newly characterized *J. regia* complete chloroplast genome will provide essential data for further study on the phylogeny and evolution of the genus *Juglans* and of the Juglandaceae, for molecular breeding, and potentially for genetic engineering.

Declaration of interest

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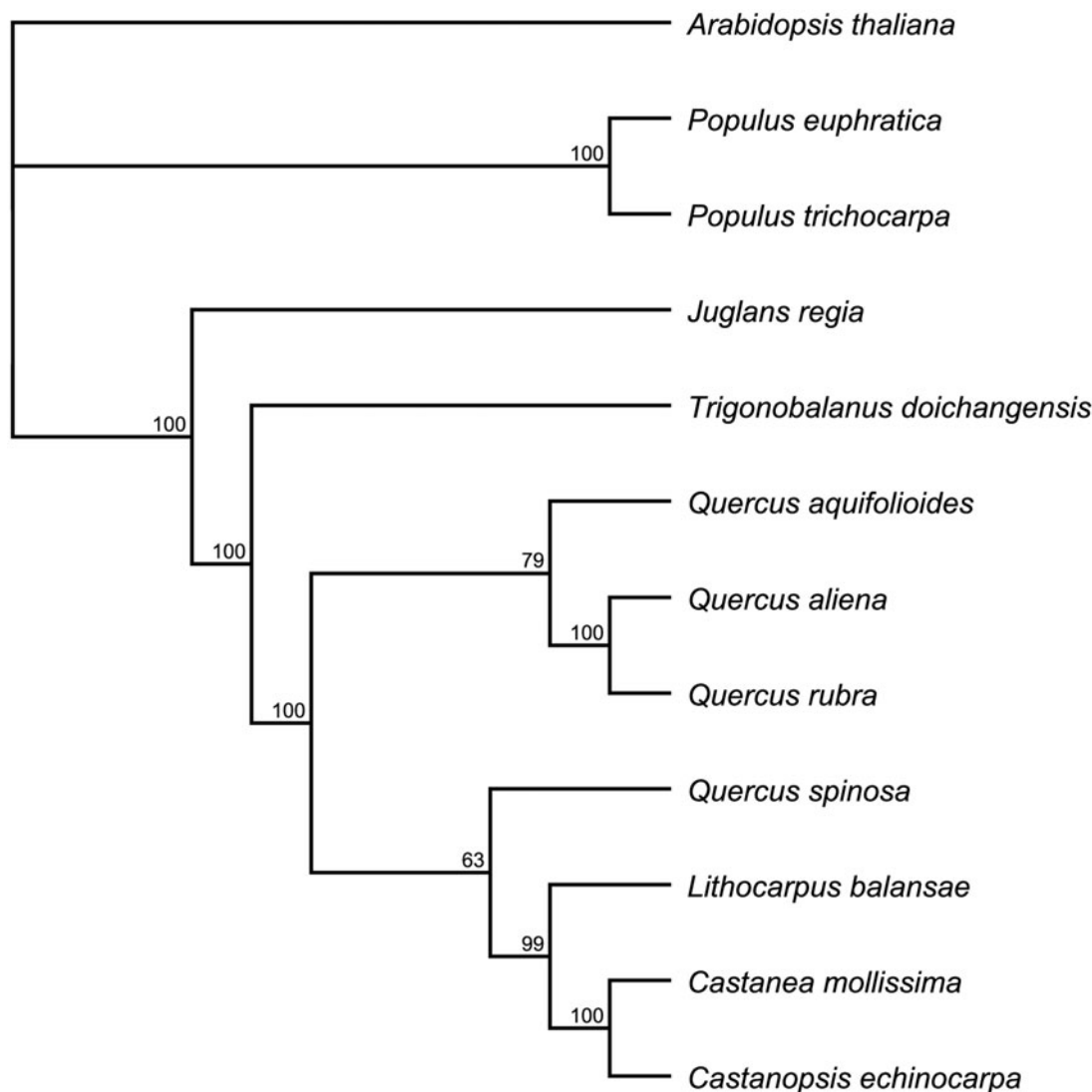


Figure 1. Phylogenetic relationships among 12 chloroplast genomes. The 12 species can be divided into three independent clades: Fagales, Salicales and Brassicales. *Arabidopsis thaliana* (Brassicales) was used as an outgroup. Bootstrap support values are given at the nodes. Chloroplast genome accession number used in this phylogeny analysis: *Arabidopsis thaliana*: NC000932; *Populus euphratica*: NC024747; *Populus trichocarpa*: NC009143; *Trigonobalanus doichangensis*: KF990556; *Quercus aquifolioides*: NC026913; *Quercus aliena*: NC026790; *Quercus rubra*: JX970937; *Quercus spinosa*: NC026907; *Lithocarpus balansae*: KP299291; *Castanea mollissima*: NC014674; *Castanopsis chinocarpa*: NC023801.

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