The complete chloroplast genome sequence and phylogenetic analysis of Ilex × Koehneana ‘Wirt L. Winn’ (Aquifoliaceae)

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ABSTRACT

Ilex × Koehneana ‘Wirt L. Winn’, an important ornamental tree, has been widely distributed in southeastern China. In this study, we assembled and characterized the complete chloroplast (cp) genome of I. Koehneana to investigate its phylogenetic relationship. The whole cp genome of I. Koehneana is 157,538 bp, which contained a large single-copy (LSC) region of 87,055 bp and a small single-copy (SSC) region of 18,429 bp, and a pair of inverted repeats (IR) of 52,054 bp. A total of 137 genes, including 90 protein-coding genes, eight rRNAs, and 39 tRNAs, were identified. Phylogenetic analysis based on 74 conserved protein-coding genes revealed that I. Koehneana is closely related to I. ‘tall boy’.

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Ilex L., in the monogenic family Aquifoliaceae, is a woody dioecious genus cultivated as ornamentals, pharmaceutical plants, and industrial materials (Su et al. 2020). Ilex × Koehneana, also known as Koehne Holly, is an artificial hybrid between I. aquifolium L. and I. latifolia Thunb. Of them, I. Koehneana ‘Wirt L. Winn’ is an evergreen tree with broadleaf, and has been widely distributed in southeastern China for its ornamental, economical, and ecological values. However, due to the similar leave and flower with other species and cultivars, it is difficult to be identified and classified by morphology (Yao et al. 2016). The chloroplast (cp) genome has been extensively applied to genetic and evolutionary studies (Freitas et al. 2018). Hitherto, the cp genome of I. Koehneana remains undocumented. In this study, we reported and characterized its complete cp genome sequence based on Illumina sequencing data and bioinformatics analysis, which will provide more informatics data for the germplasm identification and phylogeny analysis of Ilex genus.

The fresh leaves of I. Koehneana were sampled from Nanjing Botanical Garden, Mem. Sun Yat-sen (118°49′55″E, 32°3′32″N), Nanjing, China. The voucher specimen (No. NBGJIB-Ilex-0019) was preserved at the Institute of Botany, Jiangsu Province, and Chinese Academy of Science. Genomic DNA was extracted using the GMS16011.2.1 Kit (Genmed Sciences Inc., Wilmington, DE) according to the instructions. DNA quality and integrity were assessed using a NanoDrop spectrophotometer (Thermo Scientific; Waltham, MA) and agarose gel electrophoresis. Then, qualified DNA was used for library construction and sequencing, which was conducted using Nova-PE150 strategy with an insert-size of 350 bp at Novogene Company. As a result, 6768.01 Mb of raw data (4512.01 Mb clean data) were obtained. De novo cp genome assembly and annotation were conducted by NOVOPlasy version 3.3 (Dierckxsens et al. 2017) and GeSeq (Tillich et al. 2017), respectively. The annotated cp genome was deposited in GenBank database (accession no. MT435528).

The complete cp genome of I. Koehneana was 157,538 bp with 37.64% GC content, including a large single-copy (LSC) region of 87,055 bp, a small single-copy (SSC) region of 18,429 bp and a pair of inverted repeats (IRs, including IRA and IRb) of 52,054 bp. A total of 137 genes predicted, which contained 90 protein-coding genes, eight rRNAs, and 39 tRNAs. Among them, 15 splitting genes contained introns and two of them (ycf3 and clpP) had two introns.

To investigate the phylogenetic relationship of I. Koehneana, phylogenetic analysis was conducted based on other 15 Ilex complete cp genomes and one taxa from Couroupita guianensis was served as outgroup (Cascales et al. 2017; Park et al. 2019; Su et al. 2020; Yao et al. 2016). Phylogenetic tree was performed based on 74 conserved protein-coding genes with those of 17 plant cp genomes by maximum likelihood method using PhyML version 3.0 software (http://www.atgc-montpellier.fr/phyml/) (Liu et al. 2019). The bootstrap values were calculated using 1000 replicates. As shown in Figure 1, the phylogenetic tree showed that I. Koehneana cluster into Ilex section and has relatively close relationship with I. ‘tall boy’ in this phylogenetic pattern (Figure 1). The cp genome sequence of I. Koehneana will provide valuable information for further analysis on population genomic and phylogenomic studies of Aquifoliaceae.
Disclosure statement
No potential conflict of interest was reported by the author(s).

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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. MT435528. The associated BioProject numbers are SRR13245415.

References
Cascales J, Bracco M, Garberoglio MJ, Poggio L, Gottlieb AM. 2017. Integral phylogenomic approach over Ilex L. species from southern South America. Life. 7(4):47.

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45(4):e18.

Freitas A, da Anunciação R, D’Oliveira-Matielo C, Stefenon V. 2018. Chloroplast DNA: a promising source of information for plant phylogeny and traceability. J Mol Biol Methods. 1:2.

Gottlieb AM, Giberti GC, Poggio L. 2005. Molecular analyses of the genus Ilex (Aquifoliaceae) in southern South America, evidence from AFLP and ITS sequence data. Am J Bot. 92(2):352–369.

Liu J, Champer J, Langmüller AM, Liu C, Chung J, Reeves R, Luthra A, Lee YL, Vaughn AH, Clark AG, et al. 2019. Maximum likelihood estimation of fitness components in experimental evolution. Genetics. 211(3):1005–1017.

Park J, Kim Y, Nam S, Kwon W, Xi H. 2019. The complete chloroplast genome of horned holly, Ilex cornuta Lindl. & Paxton (Aquifoliaceae). Mitochondrial DNA Part B. 4(1):1275–1276.

Su T, Zhang M, Shan Z, Li X, Zhou B, Wu H, Han M. 2020. Comparative survey of morphological variations and plastid genome sequencing reveals phylogenetic divergence between four endemic Ilex species. Forests. 11(9):964.

Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq—versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45(W1):W6–11.

Yao X, Tan YH, Liu YY, Song Y, Yang JB, Corlett RT. 2016. Chloroplast genome structure in Ilex (Aquifoliaceae). Sci Rep. 6:28559–28510.

Figure 1. Maximum-likelihood phylogenetic tree based on the sequences of I. Koehneana and other 16 species. Section names were displayed in the right side of phylogenetic tree (Gottlieb et al. 2005; Su et al. 2020). Numbers on the nodes indicate bootstrap values.