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## The complete chloroplast genome of horned holly, *Ilex cornuta* Lindl. & Paxton (Aquifoliaceae)

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### ABSTRACT

*Ilex cornuta* Lindl. & Paxton is a species distributed in eastern China and Korea, utilized as traditional medical plants as well as horticultural species. Here, we completed chloroplast genome of *I. cornuta*. Its length is 157,224 bp long and has four subregions: 86,610 bp of large single copy (LSC) and 18,429 bp of small single copy (SSC) regions are separated by 26,092 bp of inverted repeat (IR) regions including 131 genes (86 protein-coding genes, eight rRNAs, and 37 tRNAs). The overall GC content of this chloroplast genome is 37.7% and those in the LSC, SSC, and IR regions are 35.7%, 31.9%, and 42.9%, respectively. Phylogenetic trees show that *I. cornuta* is clustered in Section *Ilex* clade clearly. In addition, two *Ilex* species from South America are not in a basal clade, which is different from previous phylogenetic study, suggesting more *Ilex* chloroplast genomes are required to understand phylogenetic relationships of *Ilex* species.

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*Ilex cornuta* Lindl. & Paxton, distributed only in eastern China and Korea (Hu 1949), has been utilized as a horticultural species because its leaves are distinctive rectangular foliage (one or two spines per side; Yang and Peng 2019) and its fruits are red berries (Hu 1949). Many cultivars and hybrids have been developed based on this species (Hodges et al. 2001). Together with *Ilex aquifolium* usually distributed in Europe, it is a typical species for Christmas tree inside home. In addition, it has been utilized as medical plant in China so that it contains several useful compounds (Wenjuan et al. 1986; Alikaridis 1987).

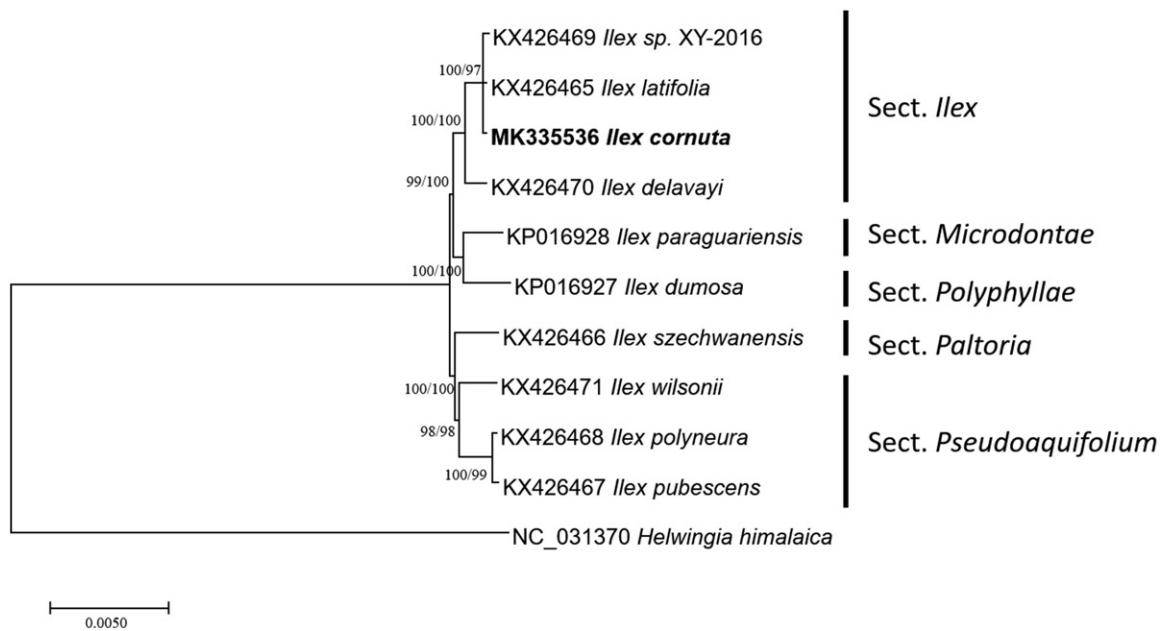
Till now, nine complete chloroplast genomes of *Ilex* were sequenced: Seven of nine species are from China (Yao et al. 2016) and two are from South America (Cascales et al. 2017). Chloroplast genome of *I. cornuta* was also analyzed one time; however, they could not complete its chloroplast genome due to lack of sample (Cascales et al. 2017).

Total DNA of *I. cornuta* isolated from Chollipo arboretum in Tae-an gun, South Chungcheong Province, Korea (Voucher in InfoBoss Cyber Herbarium (IN); S. Nam, IB-00590) was extracted from fresh leaves using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Genome was sequenced using HiSeqX at Macrogen Inc., Korea, and *de novo* assembly and confirmation were performed by Velvet 1.2.10 (Zerbino and Birney 2008), SOAPGapCloser 1.12 (Zhao et al. 2011), BWA 0.7.17 (Li 2013), and SAMtools 1.9 (Li et al. 2009). Geneious R11 11.0.5 (Biomatters Ltd., Auckland, New Zealand) was used for annotation based on *Ilex*

*paraguariensis* chloroplast, complete genome (KP016928; Cascales et al. 2017).

The chloroplast genome of *I. cornuta* (Genbank accession is MK335536) is 157,224 bp (GC ratio is 37.7%) and has four subregions: 86,610 bp of large single copy (LSC; 35.7%) and 18,429 bp of small single copy (SSC; 31.9%) regions are separated by 26,092 bp of inverted repeat (IR; 42.9%). It contains 131 genes (86 protein-coding genes, eight rRNAs, and 37 tRNAs); 18 genes (seven protein-coding gene, four rRNAs, and seven tRNAs) are duplicated in IR regions.

Ten *Ilex* and one *Helwingia* chloroplast genomes from Aquifoliaceae were used for constructing neighbor joining (bootstrap repeat is 10,000) and maximum likelihood (bootstrap repeat is 1,000) phylogenetic trees using MEGA X (Kumar et al. 2018) after aligning whole chloroplast genome sequences using MAFFT 7.388 (Katoh and Standley 2013). Phylogenetic trees show that *I. cornuta* is clustered with *Ilex latifolia* and new species of *Ilex* (Section *Ilex* in Figure 1; Yao et al. 2016), agreeing with previous studies (Cuénoud et al. 2000; Jiang et al. 2017) in Section *Ilex* (Wu et al. 2008). In addition, *Ilex dumosa* and *Ilex paraguariensis* originated from South America are not in basal clade of *Ilex* genus with high bootstrap supports (Figure 1), disagreeing with the previous study (Gottlieb et al. 2005). Due to lack of phylogenetic study of *Ilex* genus covering both Eastern Asia and South America samples at the same time, additional *Ilex* chloroplast genomes are strongly required to uncover *Ilex* phylogenetic relationship.



**Figure 1.** Neighbor joining (bootstrap repeat is 10,000) and maximum likelihood (bootstrap repeat is 1,000) phylogenetic trees of ten *Ilex* and one *Helwingia* chloroplast genomes from Aquifoliaceae: *Ilex cornuta* (MK335536 in this study), *Ilex latifolia* (KX426465), *Ilex sp. XY-2016* (KX426469), *Ilex delavayi* (KX426470), *Ilex paraguariensis* (KP016928), *Ilex dumosa* (KP016927), *Ilex szechwanensis* (KX426466), *Ilex wilsonii* (KX426471), *Ilex polyneura* (KX426468), *Ilex pubescens* (KX426467), and *Helwingia himalaica* (NC\_031370). Section names were displayed in the right side of phylogenetic tree (Gottlieb et al. 2005; Wu et al. 2008; Jiang et al. 2017). Phylogenetic tree was drawn based on neighbor joining tree. The numbers above branches indicate bootstrap support values of maximum likelihood and neighbor joining phylogenetic trees, respectively.

## Disclosure statement

The authors declare that they have no competing interests.

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