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Chloroplast characterizations and phylogenetic location of a common ornamental cherry cultivar, *Prunus campanulata* 'Kanhizakura-plena' (Rosaceae)

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ABSTRACT

Flowering cherries are well-grown in the world to develop a gorgeous landscape. Though several species are sequenced, there is huge mass of genome-level aberrances between wild species and highly domesticated cultivars. Herein, we established the complete chloroplast genome of *Prunus campanulata* 'Kanhizakura-plena'. The chloroplast genome circle (157948 bp) was formed by an 85949 bp large single-copy (LSC) region, a 19127 bp small single-copy (SSC) region, and 2 inverted repeat (IRs) regions of 26436 bp. Independent annotation showed 124 genes were found and conserved tRNA genes and rRNA genes were 37 and 8, respectively. The overall GC content was 36.72%, same with the known species, *P. campanulata*. Phylogenetic tree confirmed the relationship that *P. campanulata* 'Kanhizakura-plena' is most closely related to *P. campanulata*, nested inside Prunus. This announcement of chloroplast genome helps genetic modification and phylogenetic study in *Prunus* genus with useful information. It is a valuable resource for further breeding.

Plants in Rosaceae always gain gifts from photosynthesis. Most of the species gather enjoyable utilitarian function by producing fruit and manufacturing flowering landscape. Especially, in *Cerasus,* original species are domesticated to cultivars in order to strengthen economically and ornamentally characters. Thus, some flowering cherries have experienced multiple hybridizations. *Prunus campanulata* 'Kanhizakura-plena' is a well-improved cultivar that first bred in Taiwan, China. Its red petals come from parent *Prunus campanulate,* formerly named as *Cerasus campanulate*, and more layers of petal and bell-shaped corolla make this cultivar enjoyable to local gardeners that it has been widely promoted and the planting volume increases rapidly. This cultivar is common to most areas of China especially in Taiwan, Fujian, Guangdong, Yunnan. Recent genomes in genus *Prunus* notarize that the nuclear genome is around 300 Mb. Genomes of Cerasus × yedoensis 'Somei-Yoshino' (Shirasawa et al. 2019) and *Prunus yedoensis* display interspecific hybridization between sympatric flowering cherries (Baek et al. 2018). High-quality complete chloroplast genome (cp) helps differentiate the cultivars and focus on vital functional gene loss or gain in plastome. Comparative genome of inner or outer species in genus level also provided a new promising method for phylogeny, population dynamics, and species evolution (Li et al. 2019). Thus, it is interesting to assemble and characterize the cp genome of *P. campanulata*

'Kanhizakura-plena' to provide a better understanding of the domestication and genetics in this genus.

Plants for experiments were located in Fujian province, China (26°20′21.3″ N, 113°12′39.6″ E). Samples for experiments were collected and then preserved in the laboratory of Fujian Agriculture and Forestry University. Total genomic DNA was extracted from fresh leaves by modified CTAB method. The frozen samples including fresh tissues, specimens, and sequenced DNA can be acquired by the following voucher specimen accession number (YT-FJ2019-10A, FAFU). PE150 pair-end library strategy was adopted and sequences were obtained by the BGI-500 platform (BGI, Wuhan, China) (Mak et al. 2017). We obtained a total of about 6 Gb clean reads after removing adapters and low-quality reads by fastp software (Chen et al. 2018) and reads were corrected by bfc, a standalone high-performance error-correcting tool. Then, the processed data were assembled by GetOrganelle version 1.5.2 flow, in which core mapping software and assembly tool were bowtie2 and Spades. Random separated reads were then assembled and extended into contigs. Fragments with low sequence coverages were removed as noises during the screening progress by using Bandage version 0.8.1 and ultimately formed a complete circle chloroplast. The draft cp genome owned about a 400x coverage, clean reads were mapped to the draft cp genome to check the assembling consistency. The genome was preliminarily annotated for

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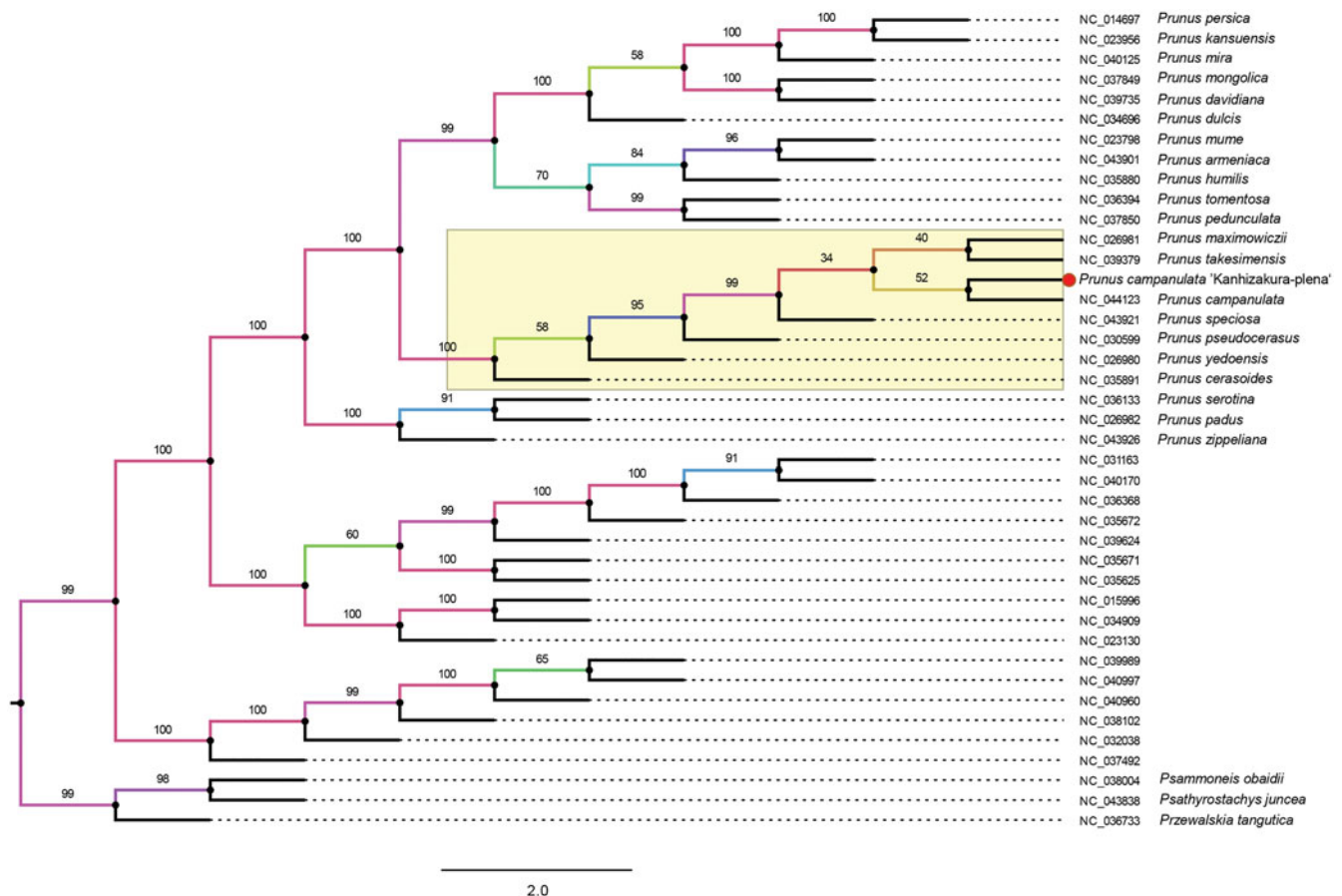
Figure 1. Maximum-likelihood (ML) phylogenetic tree with 1000 bootstraps. Outgroup is presented at the bottom. *P. campanulata* 'Kanhizakura-plena' were marked with circle.

genes and tRNA using GeSeq (Tillich et al. 2017) and tRNAscan-SE version 2.0.3 to adjust the starting position. Altogether, we established a length of 157,948 bp complete circle chloroplast genome of cultivar *P. campanulata* 'Kanhizakura-plena' with an average GC content of 36.72%. This plastid genome included a length of 85,949 bp large single-copy (LSC) region and a 19,127 bp small single-copy (SSC) region, separated by two 26,436 bp inverted repeat (IRs). The individual parts manifested unbalanced GC contents, respectively, LSC-34.59%, SSC-30.22%, and IR-42.53%. After assessment of the assembled plastid genome, we annotated the new cp-genome by online software GeSeq again. We found 124 genes, 37 tRNA, and 8 rRNA in the presented circle genome. The intact assembled chloroplast genome of *P. campanulata* 'Kanhizakura-plena' and related annotation information can be detected in GenBank (MN537437).

Members in Rosaceae have been reported mingled based on plastid phylogenomics, so as the genus *Prunus* and Cerasus, after preliminary phylogenetic proves revealed these two genera were closer in the level of morphology (Zhang et al. 2017). Therefore, we gathered the most reported chloroplast genomes in the genus *Prunus* to investigate its hybrid origin. Nearby-species complete cp genomes in Rosaceae were settled and HomBlocks pipeline was adopted to align same blocks along the plastid (Bi et al. 2018). Then, RAxML-HPC program was used to construct the maximum likelihood (ML) tree with 1000 bootstrap replicates as shown in Figure 1.

As expected in the total 41 plastids, *P. campanulata* 'Kanhizakura-plena' was more closely related to *P. campanulata* in the conventional sense of *Cerasus*, forming an independent clade in genus *Prunus*, which established an overt clustering distinction from common plums. However, there were still lots of variants in the complete plastid genome, more focus was needed on the genetic functional SNPs, which could be used as specific cultivar markers or deep into the character differences. This cluster offered proof that *Cerasus* like *Prunus campanulate* and its cultivars still owned unique positions in the genus *Prunus* in the level of complete chloroplast genome. This is mutually reflected with the traditional taxonomic results. We believe the presentation of *P. campanulata* 'Kanhizakura-plena' chloroplast genome helps clarify the evolutionary status in genus *Prunus* and provides vital genomic resources for *Prunus* breeding and fundamental researches.

Disclosure statement

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