

The complete mitochondrial genome of new species candidate of *Rosa rugosa* (Rosaceae)

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ABSTRACT

Completed mitochondrial genome of a new species candidate of *Rosa rugosa*, named as *Rosa angusta*, is 303,484 bp long. The overall GC content of this mitochondrial genome is 45.2%. It contains 52 genes covering 31 protein-coding genes, 17 tRNAs, and 3 rRNAs. In comparison to *R. rugosa* mitochondrial genome assembled from the public NGS raw reads, 124 SNPs and 769 INDELS were identified. Phylogenetic trees suggest that more *Rosa* mitochondrial genomes will be needed to understand phylogenetic relationship of the two *Rosa* species.

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Rosa rugosa, one of the firstly reported *Rosa* species in Korea, is distributed in Korea, Japan, China, and Russia (Rehder 1949). One population of *R. rugosa* was identified in 2013 by Suhwan Nam, one of the authors, showing significant differences of leaves and flowers (Kim, Heo, et al. 2019), named as *Rosa angusta* (Kim, Park, et al. 2019). Its chloroplast genome was successfully deciphered presenting 40 single nucleotide polymorphisms (SNPs) and 224 insertions and deletions (INDELS) between *R. angusta* and Chinese *R. rugosa* (Kim, Heo, et al. 2019). For understanding genetic background of this species, we successfully assembled its complete mitochondrial genome.

Its total DNA isolated from Hagampo coast, Wonbuk-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea, was extracted from fresh leaves by using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Voucher was deposited in InfoBoss Cyber Herbarium (IN; IB-90006). Genome was sequenced using HiSeqX at Macrogen Inc., Korea, and *de novo* assembly and gap-filling process were performed by Velvet v1.2.10 (Zerbino and Birney 2008) and SOAPGapCloser v1.12 (Zhao et al. 2011), respectively, and confirmation of each assembled bases and correctly assembled sequences done by BWA v0.7.17 (Li 2013), and SAMtools v1.9 (Li et al. 2009) under the environment of Genome Information System (GeIS; <http://geis.infoboss.co.kr/>; Park et al. in preparation). In addition, circular test was conducted for confirming that our mitochondrial genome is circular form using SOAPGapCloser v1.12 in the same way used in completing bacterial genome (Park et al. 2020). Mitochondrial genome annotation was conducted with Mitofy (Alverson et al. 2010) and then annotated genes were confirmed by comparing with mitochondrial

genome of *Rosa chinensis* (PDCK01000047) under the environment of Geneious R11 11.0.5 (Biomatters Ltd., Auckland, New Zealand).

The mitochondrial genome of *R. angusta* (GenBank accession is MN909970) is 303,484 bp (GC ratio is 45.2%). It contains 52 genes (31 protein-coding genes, 3 rRNAs, and 17 tRNAs). Simple sequence repeats (SSRs) were identified using the pipeline of the SSR database (SSRDB; <http://ssr.pe.kr/>; Park et al. in preparation) which has been utilized in various studies (Kim, Park, et al. 2019; doi:10.1093/jisesa/ieaa090; Lee et al. accepted; <https://www.hindawi.com/journals/ijg/2020/3236461>). In total, 1,051 SSRs of which total length is 11,706 bp (3.86%) were identified. 709 pentaSSRs and 199 hexaSSRs (86.39% in total) are classified as potential SSRs, which is similar to those of three *Dysphania* species (around 80%; Kim, Park, et al. 2019).

We also assembled *Rosa rugosa* mitochondrial genome from the public NGS raw reads (SRA accession is SRR7077019; Saint-Oyant et al. 2018), displaying that its length is 302,831 bp and 52 genes with the same configuration of those of *R. angusta* (GenBank accession is BK013300). Numbers of single nucleotide polymorphisms (SNPs) and insertions and deletions (INDELS) between two mitochondrial genomes are 124 (0.041%) and 769 (0.25%), respectively. These numbers are larger than those between two *Malus × domestica* mitochondrial genomes (NC_018554 and MN964891; Goremykin et al. 2012; Ge et al. 2020), suggesting that genetic distance between two *Rosa* mitochondrial genomes can be considered as interspecific relation. It is also consistent in those of Bryophyte species, including *Marchandia polymorpha* subsp. *ruderalis* (Kwon et al. 2019),

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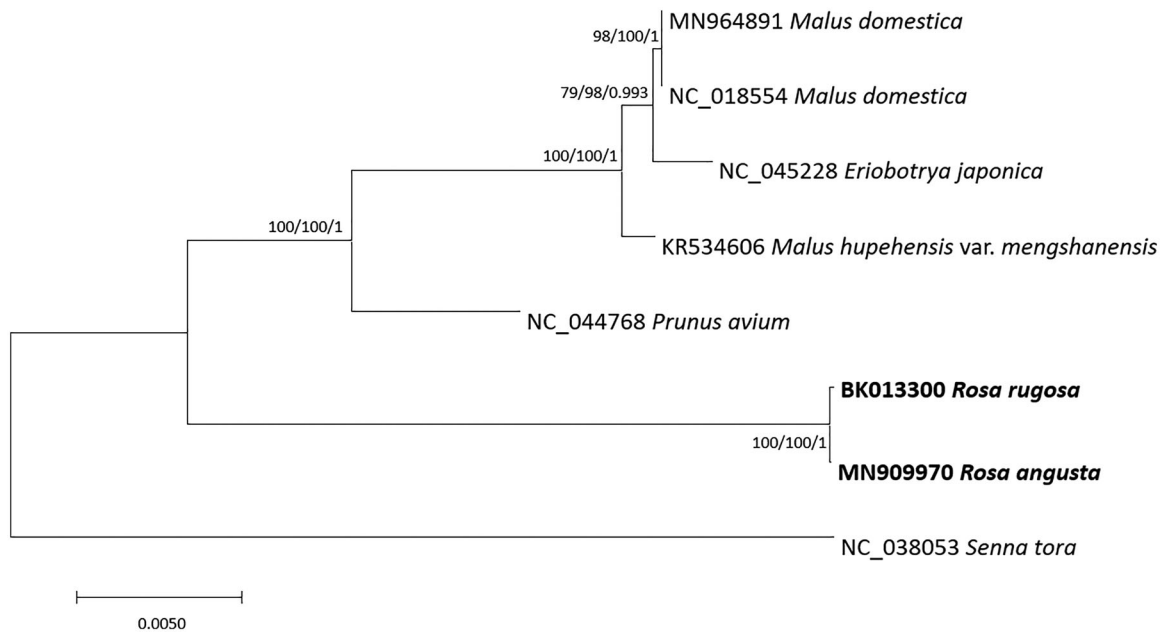


Figure 1. Neighbor-joining (bootstrap repeat is 10,000), maximum likelihood (bootstrap repeat is 1,000), and Bayesian Inference (Number of generations is 1,100,000) phylogenetic trees of 21 conserved genes (*atp1*, *atp4*, *atp6*, *atp9*, *ccmB*, *ccmC*, *ccmFN*, *cob*, *cox1*, *cox2*, *matR*, *nad1*, *nad2*, *nad3*, *nad4*, *nad5*, *nad6*, *nad7*, *nad9*, *rps1*, and *rps12*) originated from the seven mitochondrial genomes of Asteraceae and one that of Fabaceae: *Rosa angusta* (MN909970 in this study), *Rosa rugosa* (BK013300), *Malus domestica* (NC_018554 and MN964891; Goremykin et al. 2012; Ge et al. 2020), *Malus hupehensis* var. *mengshanensis* (KR534606; Duan et al. 2016), *Eriobotrya japonica* (NC_045228), *Prunus avium* (NC_044768; Yan et al. 2019), and *Senna tora* (NC_038053) as outgroup species. Phylogenetic tree was drawn based on maximum likelihood tree. The numbers above branches indicate bootstrap support values of maximum likelihood, neighbor-joining, and Bayesian Inference phylogenetic trees, respectively.

Riccia fluitans (Min et al. 2020), and *Monosolenium tenerum* (Dong et al. 2019). However, additional investigations are required because some of intraspecific variations on mitochondrial genomes, including *Liriodendron tulifipera* (Park et al. 2019) and *Arabidopsis thaliana* (Park et al. in preparation) display larger numbers.

Seven complete mitochondrial genomes in Rosaceae including those of *R. angusta* and *R. rugosa* assembled in this study and that of *Senna tora* (Fabaceae) as outgroup species, were used for constructing neighbor-joining (bootstrap repeat is 10,000), maximum likelihood (bootstrap repeat is 1,000), and Bayesian Inference (Number of generation is 1,100,000) phylogenetic trees using MEGA X (Kumar et al. 2018) and MrBayes 3.2.7a (Ronquist et al. 2012) after aligning 21 conserved genes using MAFFT v7.450 (Katoh and Standley 2013) and concatenating these alignments. We excluded one mitogenome of *R. chinensis* because its gene annotation is different from those of the other mitogenomes in Rosaceae. Phylogenetic trees show that two *Rosa* species are clustered together (Figure 1). In addition, position of *Eriobotrya japonica* is supported by slightly lower supportive values of three phylogenetic trees (Figure 1), which is congruent to the phylogenetic analysis using its whole genome (Jiang et al. 2020). Additional mitochondrial genomes of *Rosa* species will provide better result to understand phylogenetic relationship of these two *Rosa* species using mitochondrial genomes. This complete mitochondrial genome supports *R. angusta* is a new species together with its chloroplast genome sequences (Kim, Heo, et al. 2019).

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No potential conflict of interest was reported by the author(s).

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Data availability statement

The mitochondrial genome in this study can be accessed via NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) with the accession numbers of MN909970.

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