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MITOGENOME REPORT

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The complete mitochondrial genome of *Rosa laevigata* Michx. (Rosaceae), an edible and medicinal plant

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ABSTACT

In present study, the mitogenome of *Rosa laevigata* was assembled and characterized, with a total length of 281,693 bp and a GC content of 45.4%. The genome encompasses 53 genes, including 32 protein-coding genes, 3 rRNA genes and 18 tRNA genes. Furthermore, 19 MTPTs were identified, ranging from 48 to 1585 bp, covering 3.1% (8764 bp) of the mitogenome. Phylogenetic analysis of 34 Rosaceae species based on 21 common conserved protein-coding genes detected the monophyly of *Rosa*, with *R. laevigata* and *R. chinensis* forming a sister clade to *R. rugosa*. The mitogenome provides valuable genetic resources for *R. laevigata* utilization and for further phylogeny reconstruction of *Rosa* and Rosaceae.

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Introduction

The genus *Rosa* is one of the most widespread members of the Rosaceae family with more than 200 species in the temperate and subtropical regions of the northern hemisphere (Ku 1985; Gu and Robertson 2003), which is regarded as important ornamental and economic species. In addition, it also has high edible and medicinal value with a long history of application (Cunja et al. 2016; Ayati et al. 2018; Wang et al. 2022).

Rosa laevigata Michx. 1803 (Michaux 1803), commonly known as Cherokee rose, is the only species in sect. *Laevigatae* of the genus *Rosa*. It is an evergreen climbing shrub distributed throughout southern regions of China at elevations ranging from 200 to 1600 meters (Gu and Robertson 2003). It has always been a well-known edible plant with highly medicinal values and health benefits (Li et al. 2021). The fruits, roots, leaves and flowers of *R. laevigata* have been recorded as source of traditional Chinese medicines (Yuan et al. 2008; Gao et al. 2018). In the Chinese Pharmacopeia, its fruits, named 'Jin Ying Zi,' are prescribed as kidney tonic for the treatment of urinary diseases (State Pharmacopeia Commission of the Ministry of Public Health 2005). Moreover, the fruits of *R. laevigata* can also be used as health foods and food additives (He 2001; Lu et al. 2007). The Ministry of National Health of China has rated the fruit as a new food resource, and has now developed it as a third-generation wild fruit food (Li et al. 2021). Besides, the fruits contain triterpenoids, polysaccharides, flavonoids, and other compounds (Liu et al. 2010; Yan et al. 2011). The triterpenoids are the main active components and possess anti-HIV, anti-tumor, anti-Alzheimer's disease, and neuroprotective properties (Gao et al. 2018, Gao et al. 2023).

Although *R. laevigata* is of significant importance, few molecular genetic studies have been conducted on this species. Previous studies about *R. laevigata* mainly focused on traditional use, phytochemistry and pharmacology (Yan et al. 2011; Li et al. 2021; Gao et al. 2023). To date, fundamental genetic information about this species remains less, with only the chloroplast genome being reported (Zhang et al. 2019), which leads to the slow progress of germplasm resources protection and comprehensive utilization. In this study, we assembled the mitochondrial genome sequence for *R. laevigata* using Illumina short reads and PacBio long reads to further investigate its phylogenetic relationships in the genus *Rosa* and enrich the genetic resources of Cherokee roses for future utilization.

Materials and methods

Fresh young leaves of *R. laevigata* (Figure 1) were collected from Changshou District of Chongqing Municipality (107°12′53.598"E, 0°10′42.169"N, 350 m). The voucher specimens were deposited in the Herbarium of Yunnan Normal University (YNUB, Website: https://life.ynnu.edu.cn/, Contact: Jian-Lin Hang, Email: hjlynub@163.com) with the voucher number: JYZ01, which is identified by Dr. Yong- Hong Zhang. The total genomic DNA was extracted from fresh leaves using a modified CTAB (cetyltrimethylammonium bromide) method (Allen et al. 2006), followed by construction of Illumina short-read library and PacBio SMRT Bell library. Subsequently, the

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Figure 1. Photographs of *R. laevigata*. (A) Image of the flower. (B) Image of the fruit. Photos were taken by Yong-Hong Zhang.

Illumina short-read library was sequenced on the Illumina NovaSeq 6000 platform for paired-end sequencing with a read-length of 150 base pairs (bp). Concurrently, the Bell library was sequenced on the PacBio Sequel IIe platform for long-read sequencing.

A total of 52 Gb Illumina reads and 36 Gb PacBio long reads were generated, respectively. The Illumina reads were filtered by fastp v0.23.4 (Chen et al. 2018) software. The PacBio long reads were filtered by the CCS subprogram (https://github.com/PacificBiosciences/ccs). The mitogenome sequence of *Rosa chinensis* (GenBank accession number NC_065236) was selected as the reference sequence. Thereafter, the sequencing reads were mapped to the reference sequence, and the mapped reads were extracted by SAMtools v1.2 (Danecek et al. 2021) software as the candidate sequences for assembling the mitogenome of *R. laevigata*. The mitogenome of *R. laevigata* was assembled by Unicycler v0.5.1 (Wick et al. 2017) software, and Bandage v0.8.1 (Wick et al. 2015) software was used for visualization. The contigs of the mitogenome were arranged in a circular molecule using Bandage software and exported as the mitogenome sequence of *R. laevigata*. Then the online tool IPMGA (http://www.1kmpg.cn/mga/) was used to annotate it. In addition, the annotated sequence was imported into Geneious v2022.2.2 (Kearse et al. 2012) software for manual correction. Finally, mitogenome was visualized using the online software PMGmap (http://47.96.249.172:16086/home/). The annotated mitogenome was submitted to the GenBank database under accession number PQ149012.

Until now, only two mitogenomes of the genus *Rosa*, *R. chinensis* (GenBank accession number NC_065236) and *R. rugosa* (GenBank accession number NC_065237), were available. To identify the mitochondrial plastid DNAs (MTPTs), the chloroplast genome sequence (the GenBank accession numbers of the chloroplast genomes of *R. chinensis*, *R. rugosa* and *R. laevigata* are MH332770, MK641521 and NC_046824, respectively.) was searched as query in the mitogenome sequence using Blastn v2.15.0 (Chen et al. 2015) software with the e-value set to 1e-5. The detected MTPTs were annotated in Geneious v2022.2.2 software, and the results were visualized by TBtools v2.11 (Chen et al. 2020) software.

For phylogenetic analysis, the complete mitochondrial genome sequences of 35 reported species were downloaded from NCBI, including 2 species from the genus *Rosa* and 31 species from other genera of Rosaceae, with *Hemiptelea davidii* from Ulmaceae and *Hippophae tibetana* from Elaeagnaceae as outgroups. Phylosuite v1.2.3 (Zhang et al. 2020) software was used to identify and extract 21 protein-coding genes (PCGs) shared among the mitogenomes of these species and the extracted results were subjected to multiple sequence alignment analysis using MAFFT v7.490 (Katoh and Standley 2013) software. Subsequently, the maximum likelihood method (ML) phylogenetic tree was constructed using IQ-TREE v2.3.6 (Nguyen et al. 2015) software based on the aligned sequences, employing the GTR + F + I + G4 substitution model with 1000 bootstrap repetitions. The results of phylogenetic analysis were then visualized using iTOL (https://itol.embl.de/).

Results

The *R. laevigata* mitogenome was assembled into a single, circular sequence of 281,693 bp in length with a GC content of 45.4%. The reads used for assembly were mapped back to the circular mitogenome, and the average coverage depth was 4055.0× (Figure S1), indicating that the genome assembly was reliable. The mitogenome of *R. laevigata* consists of 53 genes, including 32 PCGs, 3 rRNA genes and 18 tRNA genes (Figure 2, Table S1). Among them, the PCGs contain 24 core genes present in the common ancestor of seed

plants and 8 variable genes present in extend seed plants (Mower et al. 2012). The overall length of PCGs in the mitogenome of *R. laevigata* was 29,850 bp, covering 10.6% of the genome. There are 20 introns in the mitogenome of *R. laevigata*, of which 8 are cis-splicing introns, and 12 are trans-splicing introns (Figure S2).

According to the sequence similarity analysis, 19 homologous fragments were identified in total (Figure S3), collectively spanning 8764 bp, accounting for 3.1% of the mitochondrial genome of *R. laevigata*. The shortest region was 48 bp, and the longest was 1585 bp. Altogether, 17 chloroplast genes or gene fragments were involved in these 19 MTPTs (Table S2), of which 7 were complete genes (*trnM-CAU*, *trnW-CCA*, *trnH-GUG*, *trnD-GUC*, *trnN-GUU*, *trnT-GGU*, *trnI-CAU*). In the mitogenomes of *R. chinensis* and *R. rugosa*, 18 and 21 homologous fragments were identified (Figure S4, Figure S5), respectively, involving 16 and 18 chloroplast genome genes or gene fragments (Table S3, Table S4). The total span of these fragments was 8063 bp and 11211 bp, respectively, accounting for 2.9% and 3.7% of respective mitogenomes.

Based on the 21 common conserved mitogenome PCGs (*atp1*, *atp4*, *atp8*, *atp9*, *ccmB*, *ccmC*, *ccmFc*, *ccmFn*, *cob*, *cox1*, *cox2*, *cox3*, *matR*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, *nad9*, *rps3*), a phylogenetic tree was constructed for 34 species of Rosaceae including *R. laevigata*. The sampled species within Rosaceae formed a well-supported clade with 100% bootstrap support and two sub-clades were detected with high bootstrap values. All species of the genus *Rosa* clustered together to form a monophyletic group with 100% support. Within the genus *Rosa*, *R. laevigata* and *R. chinensis* formed a

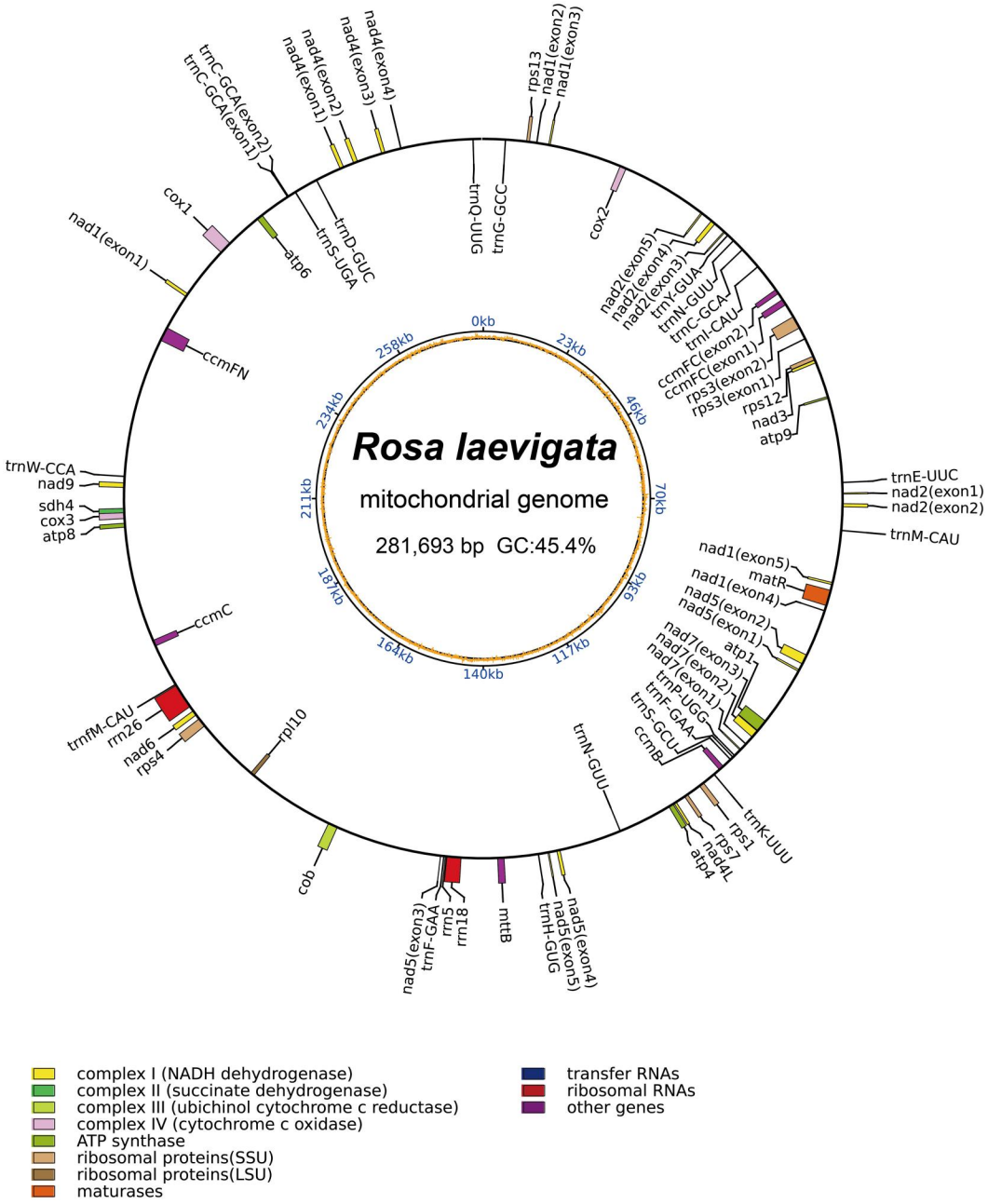

Figure 2. The circular map of the complete mitogenome of *R. laevigata*. The genome map includes three tracks. From the inward to outward, the first track depicts the GC content of the genome in orange. The second track shows the scale axis of the genome. The inner side of the third track represents the genes located on the negative strand, and the outer side represents the genes located on the positive strand. Different functional gene groups are color coded.

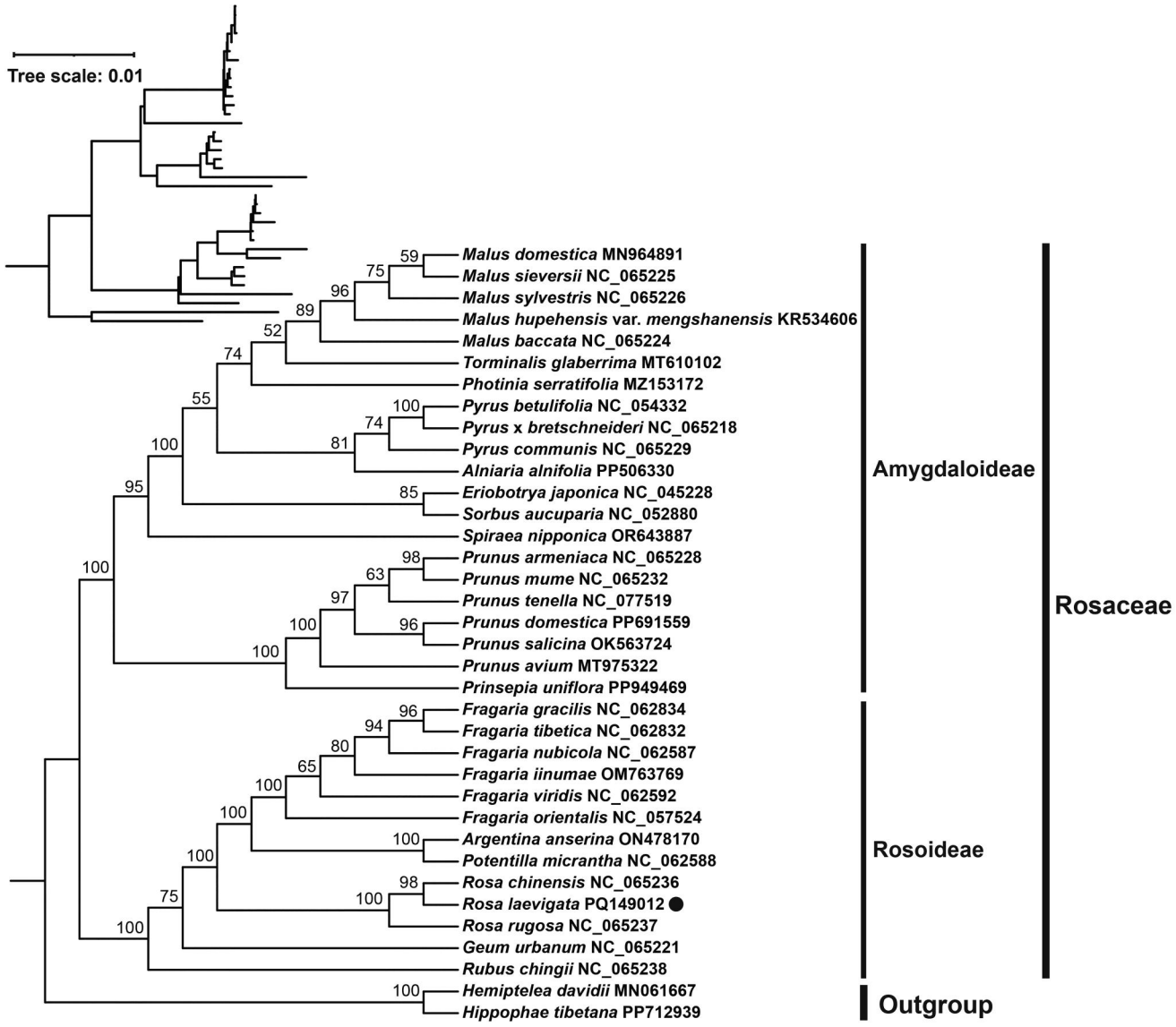

Figure 3. Maximum likelihood tree of 34 species from the Rosaceae based on based on 21 concentrated mitochondrial PCGs, with *Hemiptelea davidii* from Ulmaceae and *Hippophae tibetana* from Elaeagnaceae within the Rosales as outgroups. Bootstrap support values, based on 1000 replicates, are shown on each node. The following sequences were used: *Alniaria alnifolia* PP506330, *Argentina anserina* ON478170, *Eriobotrya japonica* NC_045228 (Yang et al. 2019), *Fragaria gracilis* NC_062834 (Fan et al. 2022), *Fragaria iinumae* OM763769 (Sun et al. 2022), *Fragaria nubicola* NC_062587 (Fan et al. 2022), *Fragaria orientalis* NC_057524 (Liu et al. 2021), *Fragaria tibetica* NC_062832 (Fan et al. 2022), *Fragaria viridis* NC_062592 (Fan et al. 2022), *Geum urbanum* NC_065221, *Hemiptelea davidii* MN061667 (Liu et al. 2019), *Hippophae tibetana* PP712939, *Malus baccata* NC_065224, *Malus domestica* MN964891 (Ge et al. 2020), *Malus hupehensis* var. *mengshanensis* KR534606 (Duan et al. 2016), *Malus sieversii* NC_065225, *Malus sylvestris* NC_065226, *Photinia serratifolia* MZ153172 (Wang et al. 2023), *Potentilla micrantha* NC_062588, *Prinsepia uniflora* PP949469, *Prunus armeniaca* NC_065228, *Prunus avium* MT975322 (Yan et al. 2019), *Prunus domestica* PP691559, *Prunus mume* NC_065232, *Prunus salicina* OK563724 (Fang et al. 2021), *Prunus tenella* NC_077519 (Liu et al. 2023), *Pyrus betulifolia* NC_054332, *Pyrus bretschneideri* NC_065218, *Pyrus communis* NC_065229, *Rubus chingii* NC_065238, *Rosa chinensis* NC_065236, *Rosa rugosa* NC_065237, *Sorbus aucuparia* NC_052880, *Spiraea nipponica* OR643887, OR643888, *Torminalis glaberrima* MT610102.

sister clade to *Rosa rugosa* with high bootstrap values (Figure 3).

Discussion and conclusion

Compared with animals, the mitochondrial genome is more complex in plants. Therefore, most of the research has been focused on chloroplasts, and the mitochondrial genome of many plants remains to be studied (Zardoya 2020). In this study, the mitogenome of *R. laevigata* was assembled and annotated for the first time. The mitogenome sizes of Rosaceae species deposited in NCBI database varies greatly, ranging from 275,143 bp (*Fragaria orientalis*: GenBank accession number NC_057524) to 535,727 bp (*Prunus mume*: GenBank accession number NC_065232). In contrast, the mitogenome size of *R. laevigata* is relatively small with 281,693 bp in length. The annotated genes of the mitogenome contain all the core genes of the angiosperm mitochondrial genome, indicating that the mitochondrial genome of *R. laevigata* is highly conserved in the gene region.

In the evolutionary process of higher plants, gene transfer between organelle genomes has always been an important phenomenon (Sloan and Wu 2014). A total of 19 homologous fragments were found in the mitochondrial and chloroplast

genomes of *R. laevigata*, including 7 complete tRNA genes, indicating that the plant mitochondrial tRNA was also derived from the migration of its own chloroplast genome sequence (Xiong et al. 2008). Among the three *Rosa* species, these MTPTs encompass eight PCGs (*psaA*, *psaB*, *rpl2*, *rpl23*, *psbC*, *psbD*, *ndhB*, *ycf1*), six intact tRNA genes (*trnW-CCA*, *trnN-GUU*, *trnI-CAU*, *trnH-GUG*, *trnD-GUC*, *trnM-CAU*), and one rRNA gene (*rrn16*). It implicated that the gene transfer between organelle genomes exhibit a high degree of similarity in the same genus.

The phylogenetic tree of *R. laevigata* and other 33 species of Rosaceae was constructed based on mitogenome information. Two sub-clades were formed within Rosaceae, which is consistent with the division of subfamilies within Rosaceae based on molecular phylogeny (Potter et al. 2007; Zhang et al. 2017). The two sub-clades represent the Amygdaloideae and Rosoideae respectively. Due to the lack of any published mitogenomes of the Dryadoideae subfamily, the phylogenetic position of this subfamily could not be solved in this study. This result is similar to the phylogenetic tree of Rosaceae constructed using orthologous genes of genome (Laczkó et al. 2024). The phylogenetic analysis also comfirmed the monophyly of the genus *Rosa*. Meanwhile, the mitogenome sequences of *Rosa* species are still limited, and the phylogenetic relationship within the genus needs further study. The mitogenome of *R. laevigata* will provide valuable resources for future phylogenetic studies of *Rosa* and Rosaceae.

Authors' contributions

Yong-Hong Zhang designed the research and revised the manuscript. Mei-Jun Zhou analyzed data and prepared a preliminary manuscript. Yi Wang and Yue Yin analyzed data and revised the manuscript. All authors read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

Ethical approval

R. laevigata is not designated as endangered species. It requires no specific permissions or licenses. No ethical approval is needed for this study.

Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) with accession number PQ149012. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1119724, SRR29286067, SRR29286022, SAMN41663458, respectively.

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