# Comparative analysis of the complete chloroplast genomes of *Mangifera* species and gene transfer between chloroplast and mitochondrial genomes

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Mango is an important commercial fruit crop belonging to the genus *Mangifera*. In this study, we reported and compared four newly sequenced chloroplast genomes of the genus *Mangifera*, which showed high similarities in overall size (157,780–157,853 bp), genome structure, gene order, and gene content. We identified three mutation hotspots (*trnG-psbZ, psbD-trnT,* and *ycf4-cemA*) as candidate DNA barcodes for *Mangifera*. We also identified 13 large fragments that were transferred from the chloroplast genome to the mitochondrial genome, and found that the similarity was more than 98%. The total size of the transferred fragment was 37,537 bp, accounting for 23.79% of the chloroplast genome. Phylogenetic analysis based on whole chloroplast genome data can provide a higher resolution for *Mangifera*.

# Comparative Analysis of the Complete Chloroplast Genomes of *Mangifera* Species and Gene Transfer Between Chloroplast and Mitochondrial Genomes

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**Abstract**

Mango is an important commercial fruit crop belonging to the genus *Mangifera*. In this study, we reported and compared four newly sequenced chloroplast genomes of the genus *Mangifera*, which showed high similarities in overall size (157,780–157,853 bp), genome structure, gene order, and gene content. We identified three mutation hotspots (*trnG-psbZ, psbD-trnT,* and *ycf4-cemA*) as candidate DNA barcodes for *Mangifera*. We also identified 13 large fragments that were transferred from the chloroplast genome to the mitochondrial genome, and found that the similarity was more than 98%. The total size of the transferred fragment was 37,537 bp, accounting for 23.79% of the chloroplast genome. Phylogenetic analysis based on whole chloroplast genome data can provide a higher resolution for *Mangifera*.

**Key words:** *Mangifera,* Chloroplast genome, DNA barcodes, Gene transfer, Phylogenetic analysis

## Introduction

Mango is a tall, evergreen tree belonging to the genus *Mangifera* of the Anacardiaceae family. It is an important tropical fruit (Iquebal et al. 2017; Lora & Hormaza 2018) that originates in tropical and subtropical regions in Southeast Asia (Dutta et al. 2013; Sherman et al. 2015). Owing to its wide range of cultivation (Bajpai et al. 2016), high nutrient value, pleasing appearance, and unique flavor, (Surapaneni et al. 2013), it is widely loved by consumers and has the reputation of being known as the "King of Tropical Fruits" (Khan et al. 2015). Southeast Asian countries have a history of mango cultivation that spans thousands of years (Ravishankar et al. 2013). Mangoes were introduced to Africa, South America, and other continents hundreds of years ago, and several varieties suitable for local cultivation have been developed (Mansour et al. 2014; Sennhenn et al. 2014). There are 69 species of mango in the world that are mainly distributed in tropical and subtropical countries including India, Indonesia, the Malay Peninsula, Thailand, and South China, of which, five species are grown in China, namely *M. indica, M. persiciformis, M. longipes, M. hiemalis,* and *M. sylvatica*; however, the varieties cultivated in production belong to *M. indica*.

Chloroplasts are special organelles that are involved in photosynthesis and consist of layers of thylakoids. They have their own DNA and can split. The chloroplast genome is conserved and consists of four parts. Two inverted repeat (IR) regions separate the small copy region (SSC) and large copy region (LSC). Currently, with the rapid development of next-generation sequencing (NGS) technology, the entire chloroplast genome has been widely used for phylogenetic analysis. They can provide a large number of variable sites for phylogenetic analysis (Gitzendanner et al. 2018). Thus, the entire chloroplast genome shows the potential to resolve evolutionary relationships and produce highly resolved phylogenetic and genetic diversity, particularly in some complex taxa or at low taxonomic levels, which have unresolved relationships (Hu et al. 2016; Huang et al. 2020; Xu et al. 2019).

In this study, the chloroplast genomes of four *Mangifera* species were sequenced and compared with *M. Indica* and 21 Sapindales plastids. The objectives of this study were as follows: (1) to comparatively analyze the chloroplast genome structure of five species of *Mangifera;* (2) to

identify highly divergent regions of the chloroplast genomes of *Mangifera*; (3) to determine the insertion of chloroplast genes into mitochondria; (4) to explore the evolutionary relationship between the genus, *Mangifera,* and Sapindales. Overall, this study would be helpful to further understand plastid evolution and phylogeny of the genus, *Mangifera*.

## Materials and methods

### Plant material, DNA extraction, and sequencing

Fresh leaves of *Mangifera* species were collected from Xishuangbanna Tropical Flowers and Plants Garden, South Yunnan, China, and frozen in liquid nitrogen. Total genomic DNA was extracted from all samples according to the method published by Li et al (Li et al. 2013). DNA quality was detected using 1% agarose gel electrophoresis and samples were stored at -80℃ until further use.

About 5–10 μg of total DNA were extracted from each of the *Mangifera* samples to construct a shotgun library with an average insertion size of 300 bp. Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA) was sequenced in the paired-end sequencing mode by Novogene Bioinformatics Technology Co. Ltd (Beijing, China), generating approximately 8.0 Gb of raw data per sample.

### Chloroplast genome assembly and annotation

Trimmomatic 0.38 was used to filter raw sequencing data (Bolger et al. 2014), and the obtained clean data were de novo assembled using SPAdes 3.61 under different K-mer parameters (Bankevich et al. 2012). The scaffolds that were positively associated with chloroplasts were arranged on the reference chloroplast genome of *M. indica* (NC_035239). Paired-end reads were remapped to consensus assembly and multiple iterations were performed to fill in the gaps in the final consensus sequence using Geneious software 2020.0.4 (Kearse et al. 2012).

Chloroplast genome annotation was performed using GeSeq to predict genes encoding proteins, transfer RNA (tRNA), and ribosomal RNA (rRNA), and was adjusted manually as needed (Tillich et al. 2017). We also manually examined the IR junctions of all *Mangifera* species. A circular diagram of the chloroplast genomes of *Mangifera* was subsequently drawn using OGDRAW 1.3.1 (Greiner et al. 2019).

### Genome comparative analysis and divergent hotspot identification

MAFFT 7.221 was used to align the chloroplast genome sequences of five *Mangifera* plants (Katoh & Standley 2013). Next, DnaSP 6.12 was used to perform a sliding window analysis with

the step size of 200 bp and window length of 600 bp, to detect the rapidly evolving molecular markers for performing phylogenetic analysis (Librado & Rozas 2009).

**Identification of chloroplast gene insertion in mitochondria**

First, we removed the BLAST hits of genes transferred between chloroplast and mitochondrial genomes by mapping the mitochondrial genome of *M. indica* (GenBank: CM021857) to the plastid genomes. Circos (Krzywinski et al. 2009) was used to map the mitochondrial and chloroplast genomes of the *Mangifera* species as well as gene-transfer fragments.

**Phylogenetic analysis**

We used the following three methods to perform phylogenetic analyses of *Mangifera* species: Bayesian Inference (BI) with a GTR + I + G model using MrBayes 3.2 while performing sample analysis of every 1000 generations (Ronquist et al. 2012), Maximum Likelihood (ML) using MEGA 7.0 with 1000 bootstrap replicates (Kumar et al. 2016), and Maximum Parsimony (MP) with a heuristic search in PAUP 4.0 with 1,000 random taxon stepwise addition sequences (Rédei 2008).

## Results and discussion

### Basic characteristics of the *Mangifera* chloroplast genomes

Raw data (approximately from $7.1 \times 10^9$ to $8.3 \times 10^9$) were obtained from *M. hiemalis* (MN917208), *M. persiciformis* (MN917209), *M. longipes* (MN917210), and *M. sylvatica* (MN917211). The four newly sequenced *Mangifera* chloroplast genomes have been presented to the NCBI.

We investigated the characteristics of four newly sequenced and one reported *Mangifera* chloroplast genomes. *Mangifera* chloroplast genome sequence sizes were 157,780~157,853 bp (Figure 1), with the largest and smallest being those of *M. longipes* and *M. indica,* respectively. *Mangifera* chloroplast genomes are characterized by a typical four-part structure, two IR copies (26354–26379 bp) separating the LSC (86673–86726 bp) and SSC (18347–18369 bp) regions. In addition, the GC content of *Mangifera* genomes was similar, ranging from 37.88–37.89%. Five *Mangifera* chloroplast genomes contained 113–115 predicted functional genes, including 79–81 protein-coding genes, four ribosomal RNA (rRNA) genes, and 30 transfer RNA (tRNA) genes (Tables 1 and 2). Furthermore, 15 functional genes, including 4–6 protein-coding genes, four ribosomal RNA genes, and seven transfer RNA gene replicate in the IR regions of the chloroplast genome. The number, type, and order of genes were found to be very similar among the five *Mangifera* chloroplast genomes (Jo et al. 2017; Rabah et al. 2017; Zhang et al. 2020). The whole chloroplast genome sequences of four *Mangifera* species were submitted to GenBank with the accession numbers of MN917208 to MN917211.

We compared the IR/SC connected regions with complete annotations and found nearly identical relative positions in the five *Mangifera* chloroplast genomes (Figure 2). All LSC-IRb connections were found to be located within the *rps19* gene, resulting in a partial expansion of the IRb region to the *rps19* gene (80–104 bp). The IRb-SSC boundary was located in the *ndhF* gene, while the SSC-IRa boundary in the five chloroplast genomes was located in the *ycf1* gene.

### Comparative *Mangifera* chloroplast genomes and Divergence Hotspot Regions

Using the comparative sequence analysis of the five species of *Mangifera*, we found that the

plastid genome was quite conservative in the five taxa, although there were a few regions with variations. In general, sequences are conserved in the coding region, and most of the detected variations are in the non-coding region. Consistent with similar studies involving other plants, the IR regions appear to be more conservative than the LSC and SSC regions (Fig. 1) (Liang et al. 2019; Song et al. 2019). We searched for nucleotide substitutions in each chloroplast genome and accordingly detected 638 variable sites (0.40%) in the five chloroplast genomes, including 489 parsimony-informative sites (0.31%).

To identify hotspots of sequence divergence, the nucleotide diversity (Pi) values within the 600 bp window of the *Mangifera* chloroplast genomes were calculated (Fig. 3). We found that Pi values varied from 0–0.033, and the three hypervariable regions (Pi > 0.02) of the five *Mangifera* chloroplast genomes were *trnG-psbZ*, *psbD-trnT*, and *ycf4-cemA*. The hypervariable regions could be ideal molecular markers to distinguish *Mangifera* from other genera.

**Characterization of gene transfer of *Mangifera* chloroplast genome to mitochondrial genome**

The mitochondrial genome of *M. indica* was obtained from GenBank and was 87,1458 bp in size, approximately 5.5 times that of the chloroplast genome consisting of 94 functional genes. We identified 13 large chloroplast genome fragments in the mitochondrial genome, including genes and intergenomic regions. These fragments ranged from 1522–5400 bp and the sequences were over 98% consistent. The total length of these fragments was 37,537 bp, accounting for 23.79% of the chloroplast genome (Fig. 4).

Intracellular gene transfer exists between different genomes, including those of the chloroplasts, mitochondria, and nuclei (Nguyen et al. 2020; Timmis et al. 2004). Research shows that the frequency of nuclear DNA transfer from organelles in angiosperms is very high (Hazkani-Covo et al. 2010; Park et al. 2014; Smith 2011). Gene transfer from chloroplast to mitochondrial genomes is a common phenomenon during long-term evolution (Gui et al. 2016; Nguyen et al. 2020). Due to high sequence similarity between the transferred chloroplast genome fragments in the mitochondrial and original chloroplast genomes, gene transfer can lead to assembly errors in these genomes.

158 **Phylogenetic relationship of chloroplast genomes**

159 In this study, we used the chloroplast genome to adjust the phylogenetic location of *Mangifera*
160 in Sapindales (Fig. 5) and performed a phylogenetic analysis of the chloroplast genome using three
161 different methods, namely, ML, MP, and BI. BI and ML analyses revealed almost the same
162 topology, and most branches had very high support (Fig. S1). However, MP trees differed slightly
163 from BI and ML trees in some taxa (Fig. S2). Despite differences between these three approaches,
164 the relationships between most groups were well resolved and highly supported, suggesting that
165 the use of chloroplast genome data does significantly improve the resolution of phylogenetic
166 analysis. Previous studies have revealed the genetic relationship of *Mangifera* through
167 morphological, nuclear, amplified fragment length polymorphism, ribosomal internal transcribed
168 spacer (ITS), and partial chloroplast gene analysis (Eiadthong et al. 2000; Nishiyama et al. 2006;
169 Sankaran et al. 2018; Yonemori et al. 2002). Phylogenetic analysis based on complete genome
170 sequences, rather than a few genes, has been carried out in a large number of higher plant species,
171 significantly improving the resolution of phylogenetic analysis (Zhai et al. 2019).

## Conclusions

In this study, the chloroplast genomes of four *Mangifera* species were sequenced and compared. It was found that the size, structure, and gene content of the *Mangifera* chloroplast genomes were conserved. Comparative analysis showed a low degree of sequence variation. We identified 13 large fragments that were transferred from the chloroplast genome to the mitochondrial genome. In addition, we identified three mutation hotspots as DNA barcodes for the identification of *Mangifera* species. These complete chloroplast genome sequences and highly variable markers provide sufficient genetic information for the phylogenetic reconstruction and species identification of the genus *Mangifera*.

## Authors' contributions

Yingfeng Niu and Jin Liu conceived of the study, wrote and revised the manuscript. Chengwen Gao performed the data analyses, and drafted the earlier version of manuscript. All authors read and approved the final manuscript.

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## Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, accession number MN917208–MN917211.

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## Tables

**Table 1 - Summary of chloroplast genome features of five *Mangifera* species.**

| Genome feature | *M. indica* | *M. longipes* | *M. persiciformis* | *M. hiemalis* | *M. sylvatica* |
|---|---|---|---|---|---|
| Total size (bp) | 157,780 | 157,853 | 157,799 | 157,796 | 157,824 |
| LSC Length (bp) | 86,673 | 86,726 | 86,724 | 86,718 | 86,719 |
| SSC Length (bp) | 18,349 | 18,369 | 18,367 | 18,368 | 18,347 |
| IR Length (bp) | 26,379 | 26,379 | 26,354 | 26,355 | 26,379 |
| Total Genes | 113 | 115 | 113 | 113 | 113 |
| Protein coding Genes | 79 | 81 | 79 | 79 | 79 |
| Structure RNAs | 34 | 34 | 34 | 34 | 34 |
| GC Content (%) | 37.89% | 37.88% | 37.88% | 37.89% | 37.89% |
| GenBank Accessions | NC035239 | MN917210 | MN917209 | MN917208 | MN917211 |

321 **Table 2 - Genes contained in *Mangifera* chloroplast genome.**

| Category | Group of genes | Name of genes |
|---|---|---|
| Self replication | Ribosomal RNA genes | *rrn4.5, rrn5, rrn16, rrn23* |
| | Small subunit of ribosome | *rps2, rps3, rps4, rps7, rps8, rps11, rps12, rps14, rps15, rps16, rps18, rps19* |
| | Transfer RNA genes | *trnR-UCU, trnS-GCU, trnA-UGC, trnC-GCA, trnF-GAA, trnG-GCC, trnG-UCC, trnD-GUC, trnE-UUC, trnH-GUG, trnN-GUU, trnP-UGG, trnQ-UUG, trnR-ACG, trnI-GAU, trnY-GUA, trnK-UUU, trnL-CAA, trnL-UAA, trnI-CAU, trnV-GAC, trnV-UAC, trnW-CCA, trnL-UAG, trnfM-CAU, trnM-CAU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU* |
| | DNA dependent RNA polymerase | *rpoA, rpoB, rpoC1, rpoC2* |
| | Large subunit of ribosome | *rpl2, rpl14, rpl16, rpl20, rpl22, rpl23, rpl32, rpl33, rpl36* |
| photosynthesis | Subunits of photosystem Ⅰ | *psaA, psaB, psaC, psaI, psaJ, ycf3, ycf4* |
| | Subunits of NADH-dehydrogenase | *ndhA, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK* |
| | Subunits of ATP synthase | *atpA, atpB, atpE, atpF, atpH, atpI* |
| | Subunits of photosystem Ⅱ | *psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ* |
| | Subunits of cytochrome complex | *petA, petB, petD, petG, petL, petN* |
| | Protease | *clpP* |
| Other genes | Maturase | *matK* |
| | Acetyl-CoA-carboxylase | |
| | c-type cytochrom synthesis gene | *ccsA* |
| | Large subunit of rubisco | *rbcL* |
| | Envelop membrane protein | *cemA* |
| | Subunit of Acetyl-CoA-carboxylase | *accD* |
| | Hypothetical chloroplast | *ycf1, ycf2, ycf15* |

322

323

## Figure legends

**Figure 1.** Sequence diagram of *Mangifera* chloroplast genomes. Gene map of *Mangifera* chloroplast genomes, sequence alignment of *Mangifera* species chloroplast genome (a: *M. Sylvatica*, b: *M. hiemalis*, c: *M. longipes,* d: *M. persiciformis* with reference to *M. indica*), GC content, and GC skew from the outside to inside.

**Figure 2.** Comparison of inverted repeat (IR) boundary among *Mangifera* species, where genes and gene fragments across IRa/b junctions are represented in color boxes above the horizontal line. Genes and IR segments are not mapped to scale.

**Figure 3.** *Mangifera* Chloroplast genomes sliding window analysis (window length: 600 bp; step size: 200 bp). X-axis: Position of a window; Y-axis: Genetic diversity per window.

**Figure 4.** Schematic diagram of gene transfer between chloroplast and mitochondria in *Mangifera* species. Colored lines within the circle show where the chloroplast genome is inserted into the mitochondrial genome. Genes within a circle are transcribed clockwise, while those outside the circle are transcribed counterclockwise.

**Figure 5.** ML phylogenetic tree of five *Mangifera* species with 21 related species in the Sapindales based on whole chloroplast genome sequence. Numbers related to the branches are ML bootstrap value, MP bootstrap value, and Bayesian posterior probability, respectively. Asterisk denotes 100% bootstrap support or 1.0 posterior probability.

## Supporting information

Additional supporting information may be found in the online version of this article.

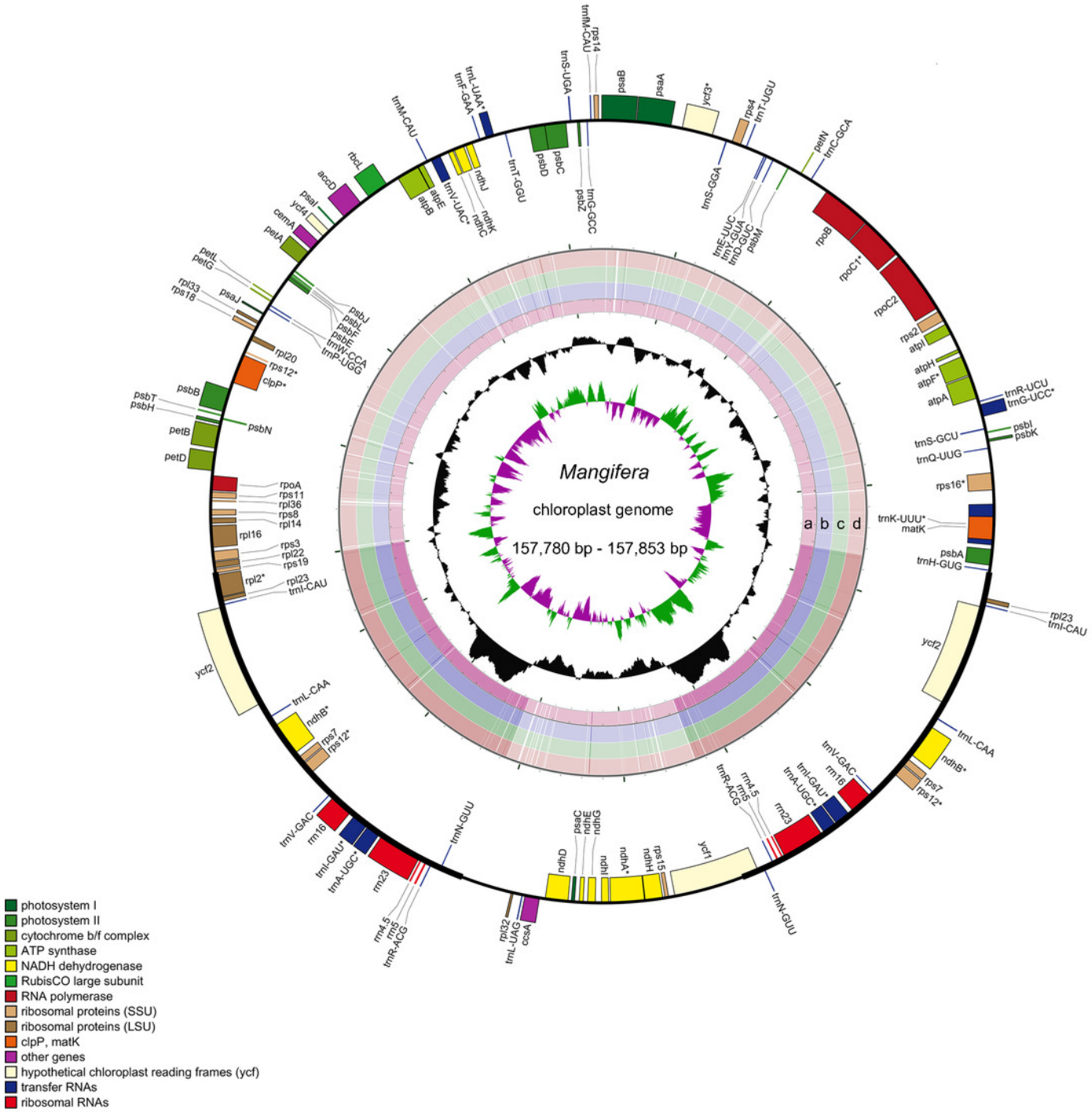
**Figure S1.** Phylogenetic trees of Sapindales based on Bayesian analysis

**Figure S2.** Phylogenetic trees of Sapindales based on maximum parsimony (MP) analysis

# Figure 1

Sequence diagram of *Mangifera* chloroplast genomes

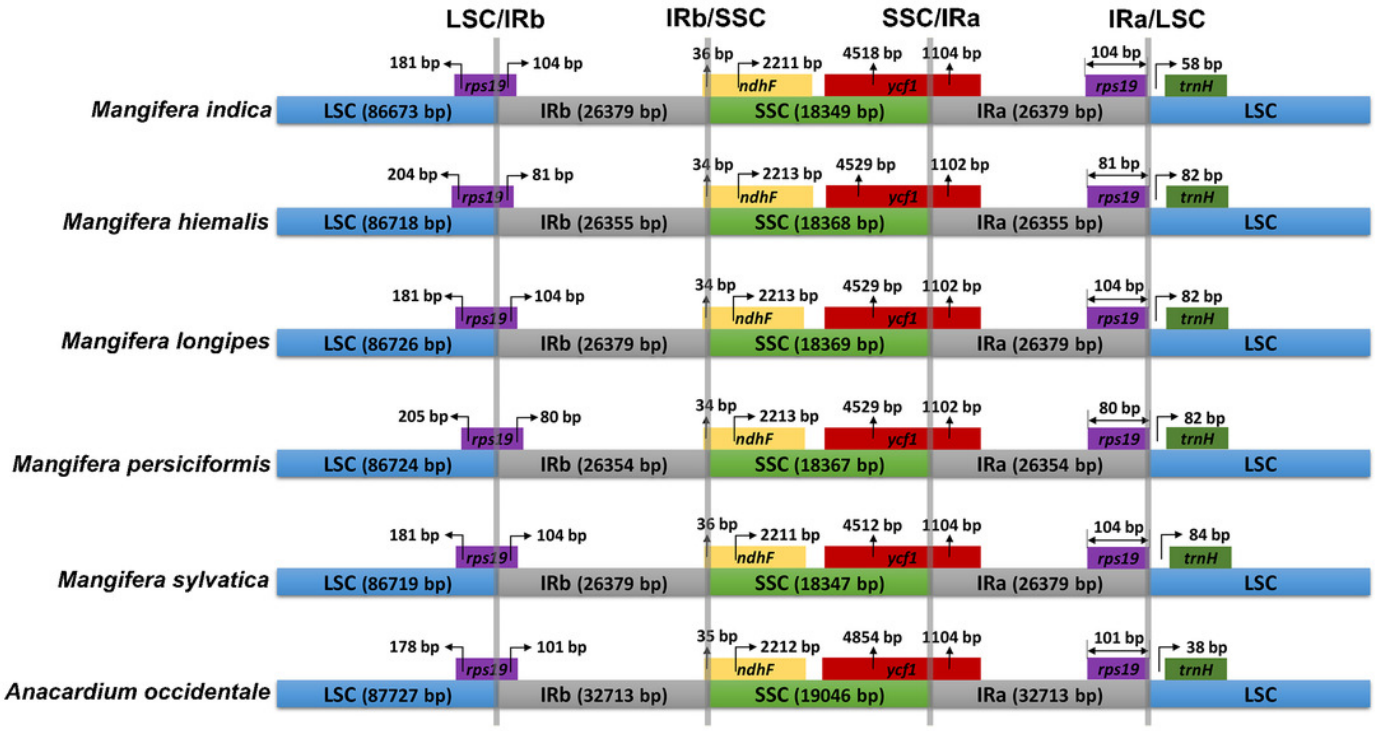
Gene map of *Mangifera* chloroplast genomes, sequence alignment of *Mangifera* species chloroplast genome (a: *M. Sylvatica*, b: *M. hiemalis*, c: *M. longipes,* d: *M. persiciformis* with reference to *M. indica*), GC content, and GC skew from the outside to inside.


Mangifera
chloroplast genome
157,780 bp - 157,853 bp
a b c d
photosystem I
photosystem II
cytochrome b/f complex
ATP synthase
NADH dehydrogenase
RubisCO large subunit
RNA polymerase
ribosomal proteins (SSU)
ribosomal proteins (LSU)
clpP, matK
other genes
hypothetical chloroplast reading frames (ycf)
transfer RNAs
ribosomal RNAs

# Figure 2

Comparison of inverted repeat (IR) boundary among *Mangifera* species

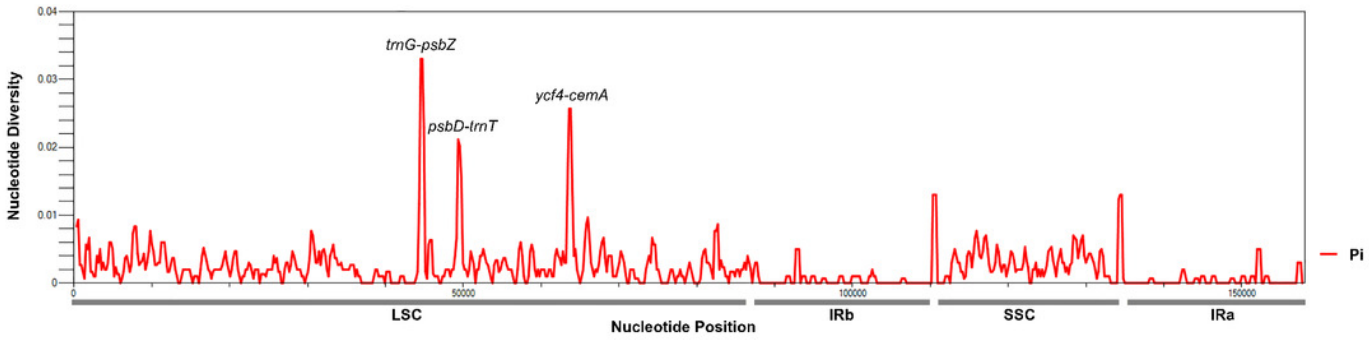
Comparison of inverted repeat (IR) boundary among *Mangifera* species, where genes and gene fragments across IRa/b junctions are represented in color boxes above the horizontal line. Genes and IR segments are not mapped to scale.



# Figure 3

*Mangifera* Chloroplast genomes sliding window analysis (window length: 600 bp; step size: 200 bp).

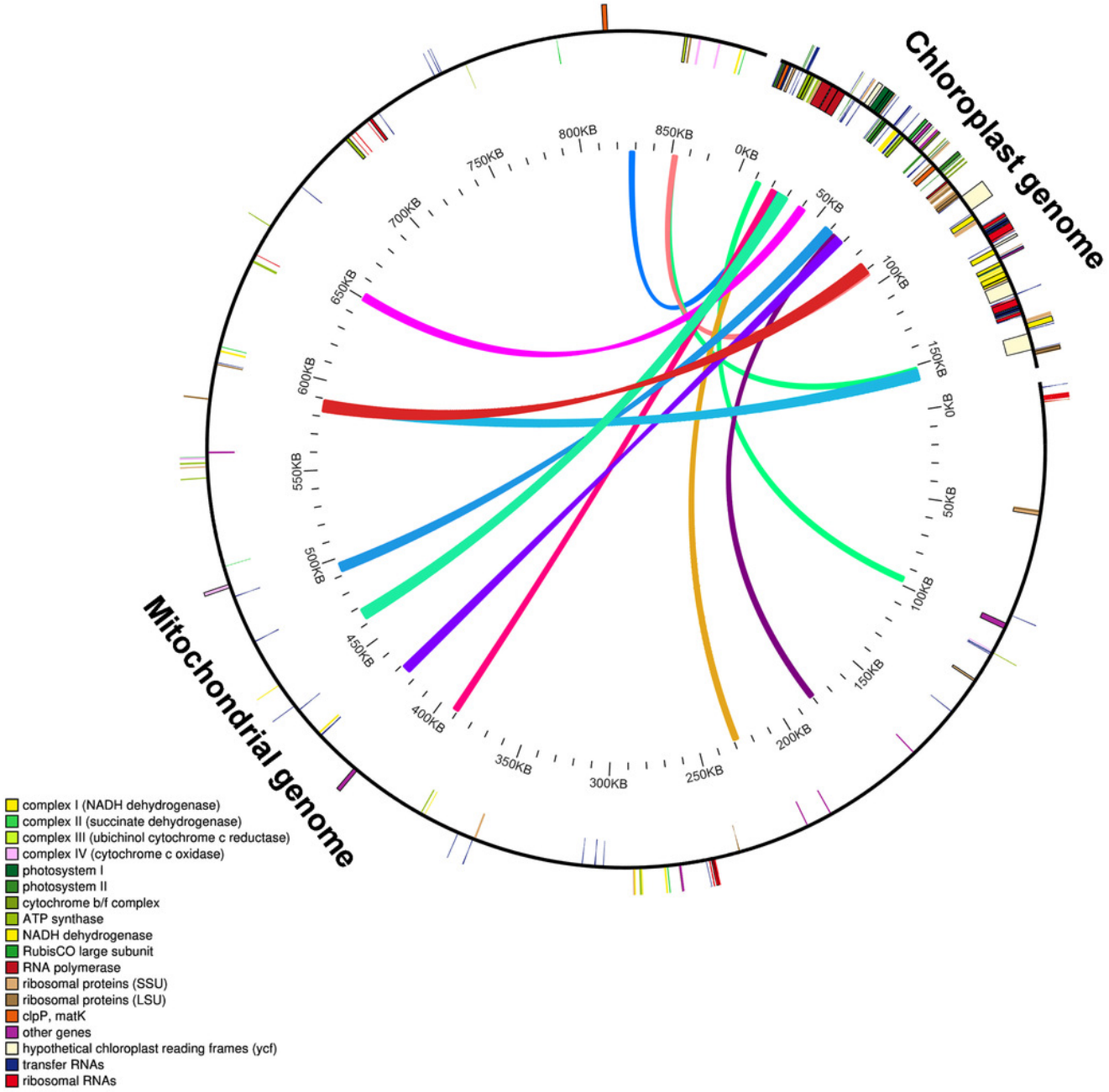
X-axis: Position of a window; Y-axis: Genetic diversity per window.



# Figure 4

Schematic diagram of gene transfer between chloroplast and mitochondria in *Mangifera* species.

Colored lines within the circle show where the chloroplast genome is inserted into the mitochondrial genome. Genes within a circle are transcribed clockwise, while those outside the circle are transcribed counterclockwise.


Chloroplast genome
Mitochondrial genome
0KB
50KB
100KB
150KB
200KB
250KB
300KB
350KB
400KB
450KB
500KB
550KB
600KB
650KB
700KB
750KB
800KB
850KB
complex I (NADH dehydrogenase)
complex II (succinate dehydrogenase)
complex III (ubichinol cytochrome c reductase)
complex IV (cytochrome c oxidase)
photosystem I
photosystem II
cytochrome b/f complex
ATP synthase
NADH dehydrogenase
RubisCO large subunit
RNA polymerase
ribosomal proteins (SSU)
ribosomal proteins (LSU)
clpP, matK
other genes
hypothetical chloroplast reading frames (ycf)
transfer RNAs
ribosomal RNAs

# Figure 5

ML phylogenetic tree of five *Mangifera* species with 21 related species in the Sapindales based on whole chloroplast genome sequence.

Numbers related to the branches are ML bootstrap value, MP bootstrap value, and Bayesian posterior probability, respectively. Asterisk denotes 100% bootstrap support or 1.0 posterior probability.