

The complete chloroplast genome of three *Syzygium* species (Myrtaceae) and their phylogenetic relationships

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Short Report

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Abstract

Syzygium cinereum (Kurz) Chantar. & J.Parn. (1993), *Syzygium cumini* (L.) Skeels (1912), and *Syzygium grande* (Wight) Walp. (1843) have different usages by humans as foods or medicines in the Southeast Asia regions. In this study, we assembled and characterized the complete chloroplast (cp) genome of three *Syzygium* species. The complete cp genomes of *S. cinereum*, *S. cumini* and *S. grande* were 158,956 bp, 158,448 bp, and 159,061 bp in length, respectively, with the overall GC content of 37.0%. All three *Syzygium* cp genomes consisted of 130 genes in total (including 85 protein-coding genes, 37 transfer RNA genes, and 8 ribosomal RNA genes). A phylogenetic tree based on cp protein-coding regions revealed the monophyly of *Syzygium* species and provided valuable insights into the phylogenetic relationships of three of our *Syzygium* species with other Myrtaceae species. The sequencing of this cp stands to expedite the development of molecular markers and significantly contribute to genetic research involving this distinctive plant.

Introduction

Syzygium Gaertn., the genus belongs to the Myrtaceae family with more than 1,200 species, most of which have a broad reported native range in tropical and subtropical climates (Craven and Biffin 2010). The genus is comprised of many species with notable usages to local people where they habit, such as food preservation, medicinal purposes, and ornamentation (Srivastava and Chandra 2013; Cortés-Rojas et al. 2014; Syahfrien et al. 2022). Furthermore, the fruits are considered exotic fruits, widely cultivated for consumption purposes, and have economic value as a product exported to other countries (Moneruzzaman et al. 2011). Their woods have high fire resistance properties and are harvested for building and construction (Datiles 2022). For example, Sea apple (*S. grande*), Malabar plum (*S. cumini*), and *S. cinereum* are the edible fruits of several *Syzygium* species well-known for their medicinal effects (Mitra 2010; Ayyanar and Subash-Babu 2012; Srivastava and Chandra 2013; Datiles 2022; Hai Nguyen et al. 2023). Furthermore, many previous studies have shown the medicinal properties of the three species, including antimicrobial (Srivastava and Chandra 2013; Cortés-Rojas et al. 2014; Sarvesan et al. 2015; Singh et al. 2016), antioxidant (Fayed et al. 2011; Srivastava and Chandra 2013; Jothiramshekar et al. 2014; Cortés-Rojas et al. 2014; Singh et al. 2016), anti-cancer activities (Fayed et al. 2011; Khodavirdipour et al. 2021), as well as being nutritious fruits (Ghosh et al. 2017).

Chloroplast is one of the most important plant organelles, whose ability to supply the energy for all bioprocesses through photosynthesis (Jensen and Leister 2014). Additionally, the cp genome is a powerful tool for both elucidating the evolutionary history of angiosperms and advancing genetic engineering applications (Bock 2014; Bennett and Triemer 2015). Recently, complete cp genomes of various *Syzygium* species have been published (63 complete cp genomes in the GenBank database, accessed on 1st June 2024) (Zhang et al. 2019; Li et al. 2021; Nguyen et al. 2023). In this study, the complete cp genomes of three *Syzygium* species (including *S. cinereum*, *S. cumini*, and *S. grande*) were characterized and used to analyze their genetic relationship within the Myrtaceae family. It is hoped that this resource will play a key role in future genetic research.

Materials

The specimens of *S. cinereum*, *S. cumini* and *S. grande* were collected from August to September 2023 in (1) Ho Chi Minh City, Vietnam (10°51'03.0"N, 106°46'18.9"E), (2) Ba Ria – Vung Tau Province, Vietnam (10°29'44.7"N, 107°13'23.9"E); (3) Ba Ria – Vung Tau Province, Vietnam (10°28'43.5"N, 107°20'29.7"E), respectively (Fig. 1). These specimens were deposited in the Department of Microbiology and Parasitology, under voucher number UMP-2023.08.34-Tramse for *S. cinereum*, UMP-2023.09.46-Trammoc for *S. cumini*, and UMP-2023.09.52-Tramdai for *S. grande* (contact person: Quang Trong Minh, email: qtminh@ump.edu.vn). All samples were dehydrated in silica gel packets and preserved at room temperature. The genomic DNA was extracted by using the DNeasy Plant Mini Kit (Cat. 69104, Qiagen).

Methods

Sequencing, assembly, and annotation cp genome

The short reads were sequenced on the MiSeq sequencer at KTEST Science Co. Ltd., Ho Chi Minh City, Vietnam (<https://www.ktest.vn/>). High-quality reads ranging from 5.02 to 5.94 Gb were obtained for each sample. Cp genome assemblies of each plant were *de novo* assembled separately using NOVOPlasty v4.3.1 to get the contigs (Dierckxsens et al. 2016). These contigs were then imported into Geneious Prime v.2023.0.4 to assemble a complete cp genome. The complete genomes were annotated using the GeSeq tool (Tillich et al. 2017). After the annotation, the cp genomes of three *Syzygium* species were uploaded onto GenBank with the accession numbers PP893285, PP893286 and PP893287 for *S. cumini*, *S. cinereum*, and *S. grande*, respectively. The map of circular cp genomes was visualized using OGDRAW (Greiner et al. 2019), and cis-splicing and trans-splicing gene maps were constructed using the CPGview website (Liu et al. 2023).

Phylogenetic analysis

To analyze the phylogenetic relationships in the Myrtaceae family, the cp genomes of three of our *Syzygium*, 67 species in the Myrtaceae family and an outgroup species from Vochysiaceae family (*Vochysia acuminata* Bong. (1839), NC_043811) were retrieved from the GenBank database (Gonçalves et al. 2019). All the species were listed in Table S1. The coding sequences of all sampled species were extracted and aligned using MAFFT v1.1.2 (Kato and Standley 2013) with default parameters. The gaps and ambiguous regions were removed by using Gblocks v0.91b (Talavera and Castresana 2007), and then sequences were concatenated to produce 73,581 nucleotide positions. The phylogenetic tree was reconstructed by using the TVM + I + G evolutionary model that was predicted by using jModelTest v2.1.10 (Santorum et al. 2014). Then, the IQ-TREE webserver was employed to reconstruct the phylogenetic tree by the maximum likelihood method with 1,000 replication bootstraps (Minh et al. 2020). FigTree v1.4.4 was utilized to edit and visualize the resulting tree (Drummond et al. 2012).

Results

The mean of coverage depths was 1161× for *S. cinereum*, 842× for *S. cumini*, and 1759× for *S. grande* (Figure S1-S3). The cp genomes of *S. cinereum*, *S. cumini* and *S. grande* were 158,956 bp, 158,448 bp and 159,061 bp in length, respectively, which exhibited typical quadripartite structure (Fig. 2). The genomes comprised of LSC (88,057–88,112 bp), SSC (18,206 – 18,810 bp) and a pair of IR (26,065 – 26,077 bp) regions. The overall GC content of the genomes was consistently around 37.0%. The region with the highest GC content was the IR regions at 42.8%; the follow-up was the LSC region with a GC content of 34.8%; and the SSC region was the region with the least GC content, which fluctuated between 30.9–31.1%. Three of the cp genomes comprised 85 protein-coding genes (PCGs), 37 tRNA genes and 8 rRNA genes (Table 1). In the IR regions, 6 PCGs (*ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12* and *ycf2*), 4 rRNA genes (*rrn5*, *rrn4.5*, *rrn23*, *rrn16*), and 7 tRNA genes (*tRNA^{Val}*, *tRNA^{Leu}*, *tRNA^{Ile}* (CAU), *tRNA^{Ile}* (GAU), *tRNA^{Asn}*, *tRNA^{Arg}*, *tRNA^{Ala}*) were duplicated. Among all genes of three *Syzygium* species, fifteen genes were shared (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps16*, *tRNA^{Val}*, *tRNA^{Leu}*, *tRNA^{Lys}*, *tRNA^{Ile}*, *tRNA^{Gly}*, and *tRNA^{Ala}*) with one intron and three genes (*clpP*, *pafl*, and *rps12*) with two introns.

Table 1
List of genes was annotated in the cp genome of three *Syzygium* species.

Groups of genes (No. of genes)	Name of genes
Ribosomal RNAs (4)	<i>rrn4.5</i> (2×), <i>rrn5</i> (2×), <i>rrn16</i> (2×), <i>rrn23</i> (2×)
Transfer RNAs (30)	<i>trnA-UGC</i> ¹ (2×), <i>trnC-GCA</i> , <i>trnD-GUC</i> , <i>trnE-UUC</i> , <i>trnF-GAA</i> , <i>trnG-UCC</i> ¹ , <i>trnG-GCC</i> , <i>trnH-GUG</i> , <i>trnI-GAU</i> ¹ (2×), <i>trnK-UUU</i> ¹ , <i>trnL-CAA</i> (2×), <i>trnL-UAA</i> ¹ , <i>trnL-UAG</i> , <i>trnM-CAU</i> , <i>trnM-CAU</i> (2×), <i>trnM-CAU</i> , <i>trnN-GUU</i> (2×), <i>trnP-UGG</i> , <i>trnQ-UUG</i> , <i>trnR-ACG</i> (2×), <i>trnR-UCU</i> , <i>trnS-GCU</i> , <i>trnS-GGA</i> , <i>trnS-UGA</i> , <i>trnT-GGU</i> , <i>trnT-UGU</i> , <i>trnV-GAC</i> (2×), <i>trnV-UAC</i> ¹ , <i>trnW-CCA</i> , <i>trnY-GUA</i>
Large units of ribosome (9)	<i>rpl2</i> ¹ (2×), <i>rpl14</i> , <i>rpl16</i> ¹ , <i>rpl20</i> , <i>rpl22</i> , <i>rpl23</i> (2×), <i>rpl32</i> , <i>rpl33</i> , <i>rpl36</i>
Small units of ribosome (12)	<i>rps2</i> , <i>rps3</i> , <i>rps4</i> , <i>rps7</i> (2×), <i>rps8</i> , <i>rps11</i> , <i>rps12</i> ² (2×), <i>rps14</i> , <i>rps15</i> , <i>rps16</i> ¹ , <i>rps18</i> , <i>rps19</i>
RNA polymerase (4)	<i>rpoA</i> , <i>rpoB</i> , <i>rpoC1</i> ¹ , <i>rpoC2</i>
Translational initiation factor (1)	<i>infA</i> ²
Subunit of photosystem I (7)	<i>psaA</i> , <i>psaB</i> , <i>psaC</i> , <i>psaI</i> , <i>psaJ</i> , <i>ycf3</i> ² , <i>ycf4</i>
Subunit of photosystem II (15)	<i>psbA</i> , <i>psbB</i> , <i>psbC</i> , <i>psbD</i> , <i>psbE</i> , <i>psbF</i> , <i>psbH</i> , <i>psbI</i> , <i>psbJ</i> , <i>psbK</i> , <i>psbL</i> , <i>psbN</i> , <i>psbM</i> , <i>psbT</i> , <i>psbZ</i>
Subunit of cytochrome (6)	<i>petA</i> , <i>petB</i> ¹ , <i>petD</i> ¹ , <i>petG</i> , <i>petL</i> , <i>petN</i>
Subunit of ATP synthases (6)	<i>atpA</i> , <i>atpB</i> , <i>atpE</i> , <i>atpF</i> ¹ , <i>atpH</i> , <i>atpI</i>
Large unit of Rubisco (1)	<i>rbcL</i>
Subunit of NADH dehydrogenase (11)	<i>ndhA</i> ¹ , <i>ndhB</i> ¹ (2×), <i>ndhC</i> , <i>ndhD</i> , <i>ndhE</i> , <i>ndhF</i> , <i>ndhG</i> , <i>ndhH</i> , <i>ndhI</i> , <i>ndhJ</i> , <i>ndhK</i>
Maturase (1)	<i>matK</i>
Envelope membrane protein (1)	<i>cemA</i>
Subunit of acetyl-CoA (1)	<i>accD</i>
(2×) duplicated gene in IR region.	
¹ gene containing a single intron.	
² gene containing two introns.	

Groups of genes (No. of genes)	Name of genes
C-type cytochrome synthesis gene (1)	<i>cssA</i>
ATP-dependent protease subunit P (1)	<i>clpP</i> ²
Component of TIC complex (1)	<i>ycf1</i>
Hypothetical proteins and conserved reading frames (1)	<i>ycf2</i> (2×)
(2×) duplicated gene in IR region.	
¹ gene containing a single intron.	
² gene containing two introns.	

The phylogenetic tree was reconstructed to observe and evaluate the relationships between three of our *Syzygium* species and related Myrtaceae taxa (Fig. 3). As a result, the phylogenetic tree revealed that *S. cinereum* was closely related to *S. nervosum*, while *S. cumini* and *S. claviflorum* had a sister relationship. Additionally, *S. grande* is revealed to be closer to *S. megacarpum* than to other *Syzygium* species.

Discussion and Conclusion

More than 1,200 species have been described in the *Syzygium* genus (Craven and Biffin 2010), but fewer than 70 complete cp genomes have been deposited in the GenBank database. In this study, we assembled and described the cp genome of three *Syzygium* species. They consisted of a pair of IRs, an SSC region, and an LSC region similar to that in the majority of other angiosperms (Jansen and Ruhlman 2012; Jensen and Leister 2014). Their genome size and gene content are consistent with previously sequenced *Syzygium* cp genomes (approximately 160 kb in length and containing 85 PCGs, 37 tRNA genes, and eight rRNA genes, respectively) (Wei et al. 2022; Nguyen et al. 2023; Sun et al. 2024). Furthermore, the phylogenetic result was correct, as the positions of *S. cinereum*, *S. cumini*, and *S. grande* followed the positions of previous research (Chakraborty et al. 2023; Nguyen et al. 2023; Sun et al. 2024). In summary, our results will contribute to enhancing the genomic data for the family Myrtaceae. The genetic resource information will be valuable for further investigations into the biology and evolutionary history of related taxa.

Declarations

Authors' contributions

TMQ, TPP: collection sample; HDN: conception and design; TPP, HDN: performed the data analysis; TMQ: drafting of the paper; HDN: critical revision of the paper. All authors read and approved the final manuscript.

Permission for sample collection

Syzygium cinereum, *Syzygium cumini* and *Syzygium grande* are not protected plants; thus, no permits are required for sample collection.

Data availability statement

The genome sequence data that support the findings of this research are openly available in GenBank under the accession number PP893285 (*S. cumini*), PP893286 (*S. cinereum*) and PP893287 (*S. grande*). The BioProject, BioSample, and SRA numbers are PRJNA1127591; SAMN42011112 (*S. cinereum*), SAMN42011113 (*S. cumini*), and SAMN42011114 (*S. grande*); SRR29517109 (*S. cinereum*), SRR29517108 (*S. cumini*), and SRR29517107 (*S. grande*).

Disclosure statement

There are no potential conflicts of interest for any of the authors.

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Figures

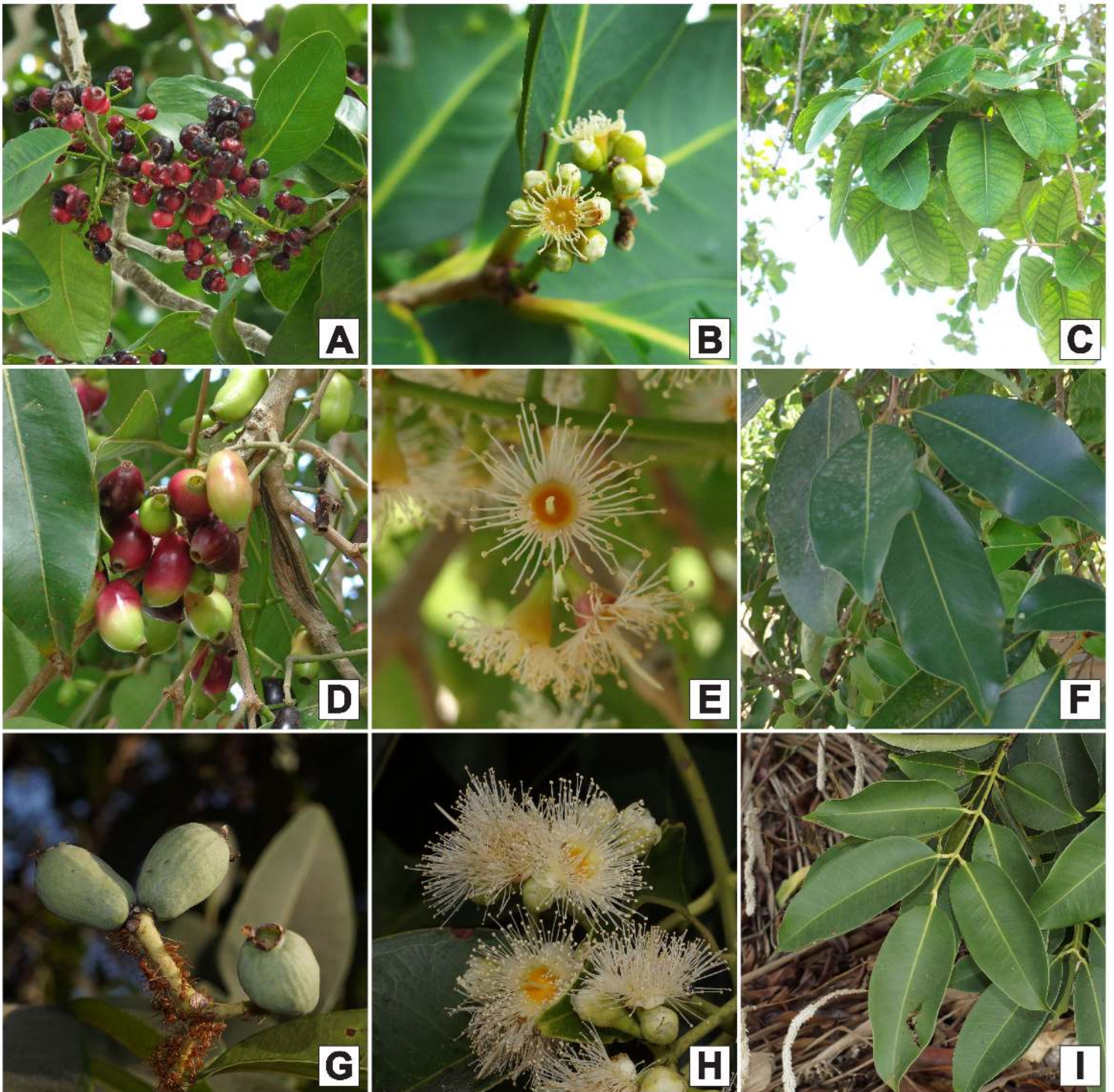


Figure 1

Photographs of *Syzygium cinereum* (fruits - A, flowers - B, and leaves - C), *Syzygium cumini* (fruits - D, flowers - E, and leaves - F), and *Syzygium grande* (fruits - G, flowers - H, and leaves - I) (photo taken by Quang Trong Minh).

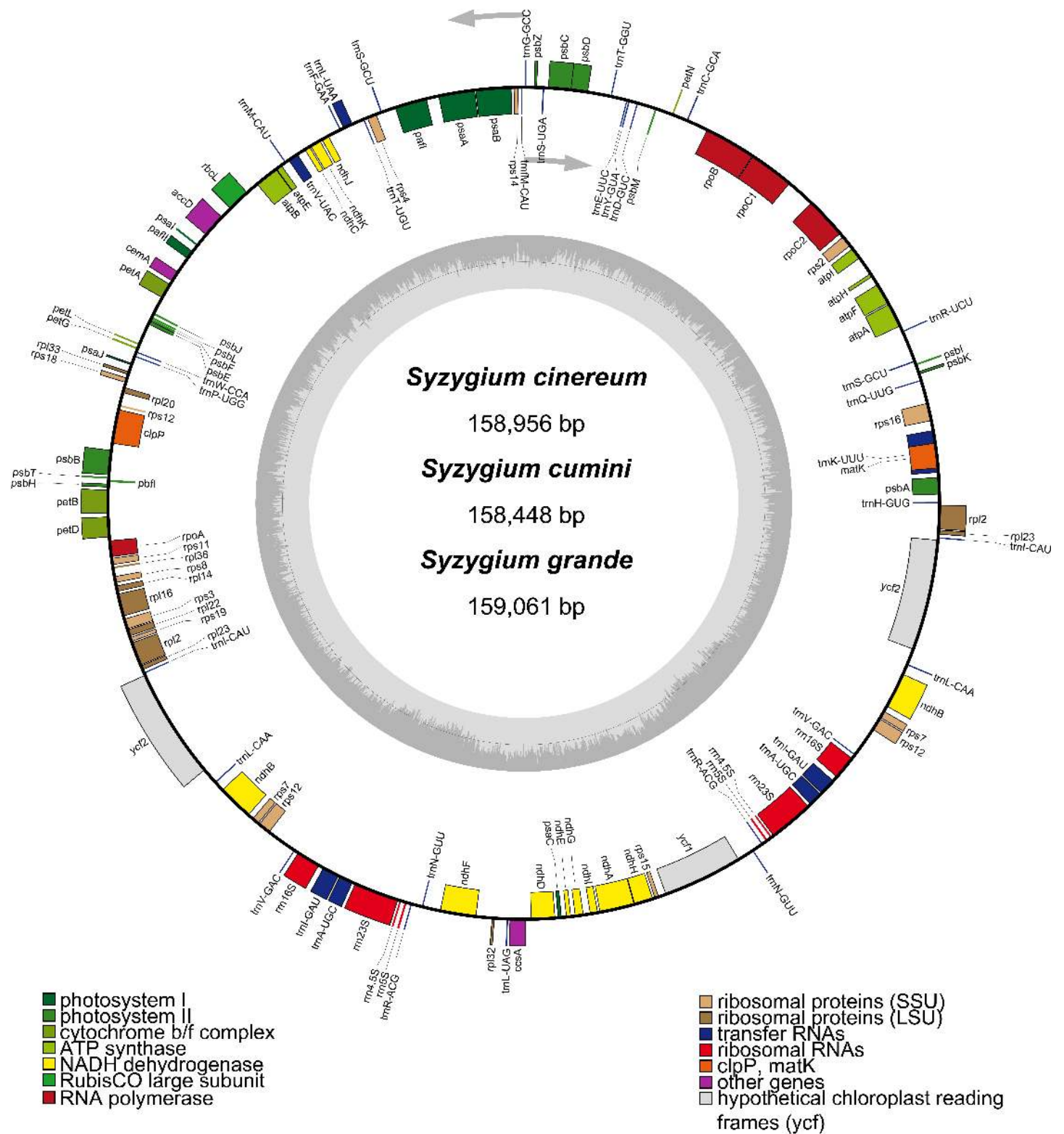


Figure 2

Chloroplast genome map of *Syzygium cinereum*, *Syzygium cumini* and *Syzygium grande*. The cp genome structure consists of a typical four-regions circular molecule with a large single-copy (LSC) region, a small single-copy (SSC) region, and a pair of inverted repeats (IRa and IRb) regions. The genes on the inside of the cp genome are transcribed in a clockwise direction, whereas the genes on the outside are transcribed in a counterclockwise direction. The inner circle consists of two parts: the dark gray part

represents the GC contain and the light gray part represents the AT contain. The functional classification of genes is signed by color-coded that is shown in two columns.

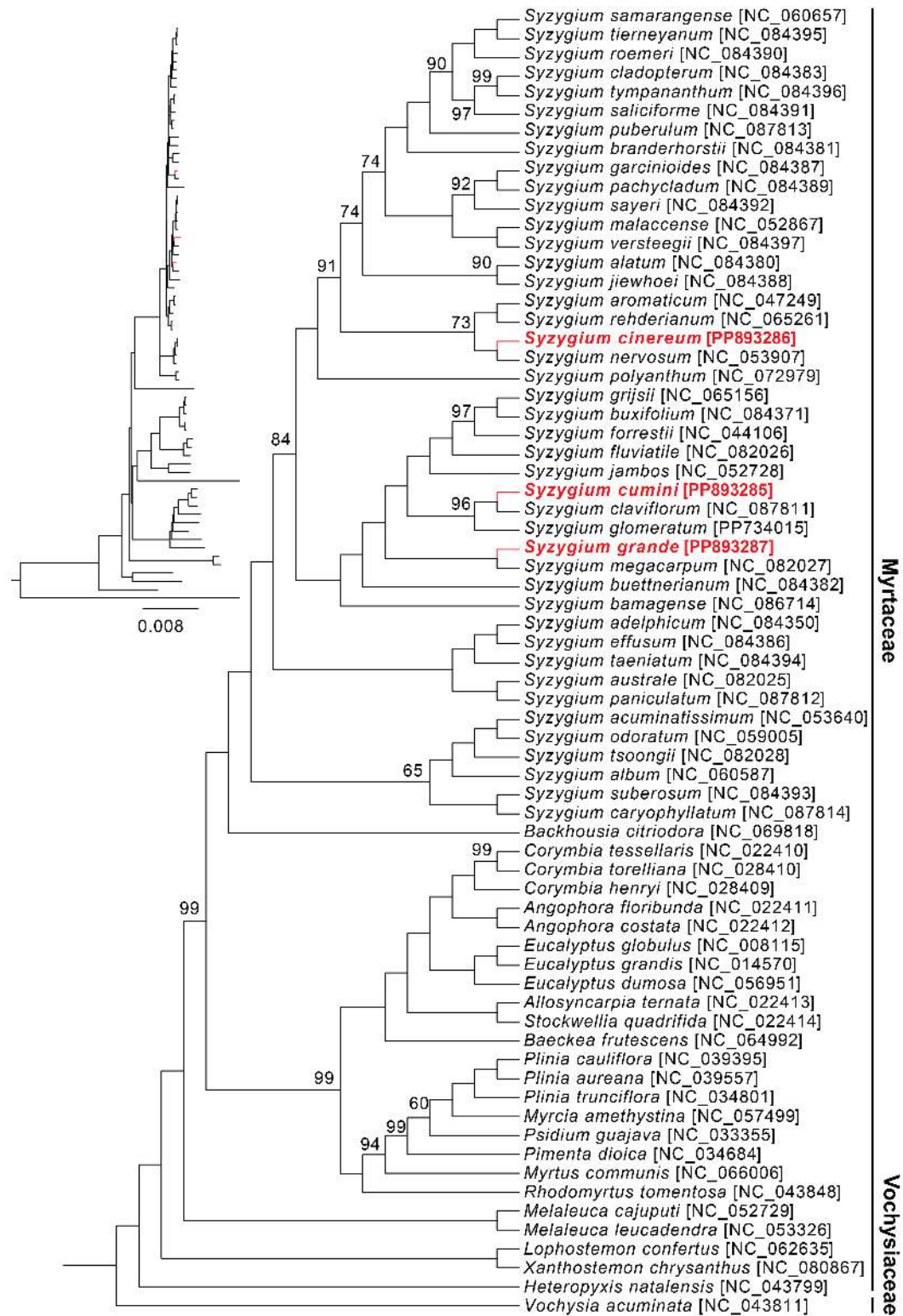


Figure 3

Phylogenetic tree based on cp protein-coding genes from 64 Myrtaceae species using maximum likelihood method.

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