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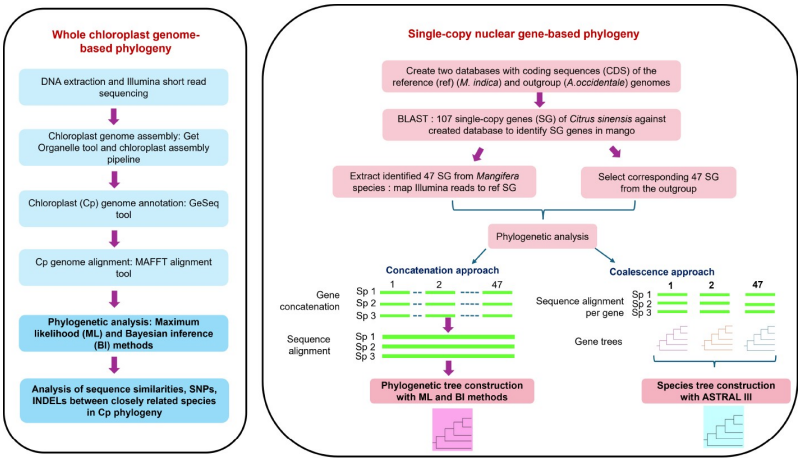
In Brief

Phylogenetic relationships among *Mangifera* species based on chloroplast and nuclear genes.

Graphical abstract



Highlights

- All *Mangifera* species had same gene number and order in the chloroplast genome.
- Phylogenies based on nuclear genes and chloroplast genomes were discordant.
- Topological incongruences suggest possible inter-specific hybridization in mango.
- *M. indica* and four wild relatives were closely related.
- Evidence of gene flow between species suggests hybrids to be common within the genus.

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Phylogenetic relationships in the genus *Mangifera* based on whole chloroplast genome and nuclear genome sequences

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Abstract

The genus *Mangifera* (*Anacardiaceae*) includes 69 species with *Mangifera indica* L. being the most important and predominantly cultivated species for commercial mango production. Although the species are classified based on morphological descriptors, molecular evidence has proposed the hybrid origin of two species suggesting the possibility that more of the species may be of hybrid origin. To analyze evolutionary relationships within the genus, 19 samples representing 14 *Mangifera* species were sequenced. Whole chloroplast genomes and 47 common single-copy nuclear gene sequences were assembled and used for phylogenetic analysis using concatenation and coalescence-based methods. The chloroplast genome size varied from 151,752 to 158,965 bp with *M. caesia* and *M. laurina* having the smallest and largest genomes, respectively. Annotation revealed 80 protein-coding genes, 31 tRNA, and four rRNA genes across all the species. Comparative analysis of whole chloroplast genome sequence and nuclear gene-based phylogenies revealed topological conflicts suggesting chloroplast capture or cross-hybridization. The chloroplast genomes of *M. altissima*, *M. applanata*, *M. caloneura*, and *M. lalijiwa* were similar to those of *M. indica* (99.9% sequence similarity). Their close sequence relationship suggests a common ancestry and likely cross-hybridization between wild relatives and *M. indica*. This study provides improved knowledge of phylogenetic relationships in the genus *Mangifera,* indicating extensive gene flow among the different species, suggesting that hybridization may be common within the genus.

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Introduction

Mango (*Mangifera indica* L.), an evergreen dicotyledonous angiosperm often referred to as the 'king of fruits' has adapted to grow in tropical and sub-tropical regions of the world[1–3]. It is considered one of the most economically successful fresh fruits cultivated in more than 100 countries. India leads the global mango production producing approximately 24.7 million tonnes accounting for 45% of total mango production followed by Indonesia (6.6%), Mexico (4.3%), China (4.3%), and Pakistan (4.3%)[4]. Besides being consumed fresh, ripe, and unripe mangoes are used to produce pickles, chutney, juices, cereal flakes, sauce, and jam building high demand for mangoes on the international market.

The taxonomic lineage of the genus *Mangifera* (*Anacardiaceae*) reveals consistent recognition of two major groups with the number of species reported varying between 45–69[1,5,6] The most accepted classification [6] defines 69 species mainly based on morphological descriptors of reproductive tissues. Of the 69 species, 58 are divided into two subgenera, *Mangifera* and *Limus*, with the remaining 11 species placed in an uncertain position due to insufficient voucher material. The subgenus *Mangifera* includes 47 species further divided into four sections: *Marchandora* Pierre, *Euantherae* Pierre, *Rawa* Kosterm, and *Mangifera* Ding Hou. *Mangifera* Ding Hou is the largest section in the genus with more than 30 species including domesticated mango (*M. indica*)[6,7]. The 11 species in sub genus *Limus* are further divided into two sections: *Deciduae* and *Perrennis*.

Due to the high demand for mango globally, systemic breeding programs have been initiated recently to develop cultivars with high productivity, improved consumer, and transportability traits. However, breeding is time-consuming due to the long juvenile period, high heterozygosity, and polyembryony observed in mango. Currently, while *M. indica* stands as the primary cultivated species for commercial fruit production, with a set of selected commercial cultivars dominating the crop improvement programs, 26 other species have been reported to produce edible fruits[7–9]. Many wild species exhibit potential significance in trait-specific breeding due to their favorable traits related to fruit quality, biotic and abiotic stress tolerance and potential as rootstocks[10–12]. Effective exploitation of these species relies on a comprehensive understanding of their distinctive characteristics within a genetic framework, as they are primarily described based on morphological traits. Therefore, identifiying molecular evolutionary relationships within the genus *Mangifera* is vital to facilitate the efficient use of wild relatives in future breeding programs.

Recent studies have used molecular markers within the chloroplast genome[10,13–15] and a set of nuclear genes[16–18] to analyze phylogenetic relationships in mango. However, the results are inconsistent, and many studies were unsuccessful in inferring evolutionary relationships with fully resolved phylogenies. Two studies have used whole chloroplast genome[19] and mitochondrial genome[17,20] sequences alone with a small

number of taxa. However, in most angiosperms, chloroplast, and mitochondrial genomes are maternally and paternally inherited, respectively[21]. Consequently, such studies prevent precise analysis of evolutionary relationships due to the use of uniparentally inherited genetic information for phylogenetic analysis.

The genus *Mangifera* is native to South and South-East Asia ranging from Indochina, Burma, Thailand, and the Malay Peninsula to Indonesia and the Philippines where some of the species are found only in the wild while others are locally grown in gardens and orchards[6]. With the introduction of the common mango to South-East Asia during the 4th–5th centuries[22], *M. indica* and wild *Mangifera* species in the region might have come into contact with each other. Since both wild and domesticated mangoes are assumed to be self-incompatible, hybridization is expected among these outcrossing species when grown in close proximity. Among wild species, a hybrid origin has been reported for *M. odorata*[23], and *M. casturi*[24,25]. With molecular data suggesting the potential of cross-hybridization in the genus, more hybrids can be expected among these 69 *Mangifera* species that have been currently identified as distinct species. Comparative phylogenetic analysis based on both chloroplast genome and ideally, a set of single-copy nuclear genes, together representing maternal and biparental inheritance respectively will be a useful approach for the precise determination of evolutionary relationships[26].

The availability of a suitable and precise reference genome is crucial in evolutionary studies to determine relationships among the species with higher accuracy. The first draft genome for *M. indica* was assembled for the Indian cultivar Amrapali[27,28]and with the use of advanced sequencing platforms, a high-quality chromosome-level genome was developed for the cultivar 'Alphonso'[29]. The genetics and genomics of chloroplasts have progressed rapidly with the advent of high-throughput sequencing technologies. Chloroplast genomes in higher plants are typically double-stranded and organized into conserved quadripartite structure, consisting of a pair of inverted repeats (IR) separated by small single copy region (SSC) and a large single copy region (LSC). The chloroplast genome size, although far smaller than most of the plant nuclear genomes ranges from 120 to 160 kb[5] with 110 to 130 genes. Conflicts between the chloroplast and nuclear phylogenetic analysis provides valuable insights into speciation, hybridization and incomplete lineage sorting[30,31]. So far, assembled chloroplast genomes of only six out of 69 species[19,32] are available.

In this study, sequences of chloroplast genomes were compared[33], and a selected set of common single-copy genes present in the nuclear genome of 14 *Mangifera* species used to analyze evolutionary relationships in the genus.

Materials and methods

Plant material and DNA extraction

Nineteen samples belonging to 14 *Mangifera* species were selected (Table 1). Leaf tissues of *M. foetida*, *M. sylvatica*, *M. quadrifida*, and one of the *M. altissima* and *M. laurina* species were sourced from The Botanical Ark, Mossman (16°22'21" S, 145°19'23" E), Queensland, Australia. *M. caesia* leaves were sourced from a tree at Fruit Forest Farm (www.fruitforestfarm.com.au), East Feluga, (17°53'46.0" S and 145°59'38.0" E), Queensland, Australia and leaves of two *M. pajang* species were sourced trees located at Treefarm, El Arish (–17°47'59.99" S and 146°00'0.00" E) and Durian Heaven Farm, Japoonvale (17°43'36" S, 145°54'35" E), Queensland, Australia. All *M. indica* varieties and other *Mangifera* species were sourced from trees grafted onto *M. indica* cv. Kensington Pride rootstock at the Walkamin Research Station, Mareeba, (17°08'02" S and 145°25'37" E), North Queensland, Australia. DNA extraction was carried out from fine pulverized mango leaf tissue samples using a cetyltrimethylammonium bromide method[34]. The quality and quantity of DNA were assessed for acceptable absorbance ratios (ideal 1.8–2.0 at A260/280 and over 2.0 at 260/230) using a Nanodrop Spectrophotometer. DNA degradation and quantity were assessed by resolving sample and standard DNA by agarose gel electrophoresis. The isolated DNA was subjected to next-generation short read sequencing on an Illumina HiSeq 2000 platform at the Ramaciotti Centre for Genomics, University of New South Wales, Australia (Supplemental Table S1).

Chloroplast genome assembly

In addition to generating sequence data for the 14 *Mangifera* species, publicly available Illumina sequencing paired-end reads were downloaded from the National Centre for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) for five *Mangifera* species namely *M. sylvatica*, *M. odorata*, *M. persiciformis*, *M. hiemalis* and *M. indica* cv. Tommy Atkins (Supplemental Table S2). Chloroplast genomes for each species were assembled using two methods: a chloroplast assembly pipeline (CAP)[35] in CLC Genomic Workbench (CLC-GWB) software (CLC Genomics Workbench 20.0, www.clcbio.com) and 'Get Organelle' pipeline (http://github.com/Kinggerm/GetOrganelle)[36]. Raw reads for all the species were imported to CLC-GWB and trimmed using the quality score limits of 0.01. The CAP processed two approaches to assemble the chloroplast genome, a reference-guided mapping approach and a *de-novo* assembly approach. For the reference guided mapping, *M. indica* cv. Tommy Atkins chloroplast genome (Accession: NC_035239.1)[37] was used as the reference. The two chloroplast sequences generated using the two approaches of the CAP for each species were aligned in Geneious 2022.2.2 software (www.geneious.com) and Clone Manager Professional 9 to identify mismatches. Manual curation of mismatches involved observing the reads mappings at the position of the mismatch. *De-novo* assembled chloroplast genomes from Get Organelle pipeline were checked in Bandage v. 0.8.1[38] to visualize the completeness of the assembled genomes. The final chloroplast genome assembled from CAP and Get Organelle pipeline were compared for mismatches and further manual curation, ensuring high-quality chloroplast genomes were assembled for all the species.

Chloroplast genome annotation and identification of single nucleotide polymorphisms (SNPs), insertions, and deletions (INDELs)

Genome annotations were performed using GeSeq tool (https://chlorobox.mpimp-golm.mpg.de/geseq.html) and *M. indica* cv. Tommy Atkins (Accession: NC_035239.1) was used as the reference. Based on the phylogenetic relationships observed in chloroplast phylogeny, closely related species within the main clades and subclades were compared to determine their evolutionary relationships. The chloroplast genomes of the species were subjected to pairwise alignment in Geneious using the MAFFT alignment tool (MAFFT v7.490)[30],

Table 1. Details of the 14 *Mangifera* species used in this study including country of origin, native distribution and important characteristics.

Sub genera	Section	Species/taxon	Embryony	Country of origin	Geographical distribution of the species	Ploidy level (2n)	Prominent fruit/horticultural characteristics
Mangifera	*Mangifera* Ding Hou	*M. laurina*	Polyembryonic	Indonesia	Wild distribution: Myanmar, Vietnam, Malesia, Thailand. Cultivated in Borneo, Sumatra, Java, (Indonesia), and the Philippines.	40	Juicy, very acid and fibrous fruit: edible. Resistance to anthracnose (fruit skin). Used as a rootstock for *M. indica* in Malaysia.
Limus	*Deciduae*	*M. pajang*	Monoembryonic	Indonesia	Endemic to Borneo (Brunei Indonesia, Malaysia). Common in cultivation.	Unknown	Fruit flesh: deep-orange-yellow, fibrous, acid to acid sweet, mildly fragrant: edible. Largest fruit in the genus.
Mangifera	*Euantherae* Pierre	*M. caloneura* (Xoài Quéo)	Polyembryonic	Vietnam	Dry deciduous dipterocarp forests in Myanmar, Thailand, Laos, and Vietnam	40	Fruit with good aroma, sour taste: edible. Drought tolerance / resistance to anthracnose (fruit skin) and gummosis.
Limus	*Perrennis*	*M. odorata*	Polyembryonic	Malaysia	Never been observed in the wild. Origin is unknown, primarily cultivated in Guam, Philippines, Thailand, and Vietnam. Introduced to Indonesia, Malaysia, and Singapore.	40	Firm, fibrous, sweet to acid-sweet, juicy fruit with a strong smell and turpentine flavour: edible. Resistance to anthracnose.
Limus	*Perrennis*	*M. foetida*	Monoembryonic	Malesia	Western part of Malesia (Sumatra, Java, Borneo, Malay Peninsula), wild and cultivates, introduced to Burma, Indochina.	40	Fruit with strong turpentine smell, sweet and pleasant, very fibrous: edible.
Mangifera	*Mangifera* Ding Hou	*M.altissima*	Polyembryonic	Philippines	Native to Philippines, Indonesia, Papua New Guinea, Solomon Islands.	Unknown	Smooth fibrous or non-fibrous fruit: edible.
Limus	*Deciduae*	*M. caesia*	Monoembryonic	Indonesia	Natural distribution: Peninsular Malaysia, Borneo (Brunei Darussalam, Indonesia, Malaysia) and Sumatra (Indonesia). Cultivated from Peninsular Thailand, Bali, Java and to the Philippines.	40	Sweet flesh with strong fragrance in the fruit: edible.
Mangifera	*Mangifera* Ding Hou	*M. zeylanica*	Monoembryonic	Sri Lanka	Endemic to Sri Lanka, not found under cultivation.	Unknown	Very juicy fruit, fibrous, pleasant, sweet taste pulp: edible.
Mangifera	*Mangifera* Ding Hou	*M. sylvatica*	Polyembryonic	Myanmar	Native to India (Sikkim), China, Myanmar, Thailand, Bangladesh, and Nepal.	40	Fruits almost fibreless, little pulp, sweet and sour taste: edible.
Mangifera	*Mangifera* Ding Hou	*M. quadrifida*	Unknown	Malesia	Sumatra, Malay Peninsula, Borneo, and Sunda Islands.	Unknown	Fruits with less fibres, sweet and pleasant smell.
Mangifera	*Mangifera* Ding Hou	*M. lalijiwa*	Polyembryonic	Indonesia	Indonesia (Java, Madura, Bali and probably in Sumatra). Very rare both in wild and in cultivation.	Unknown	Small glossy yellowish fruit with acid-sweet taste: edible.
Mangifera	*Mangifera* Ding Hou	*M. applanata*	Polyembryonic	Malesia	Native to Indonesia (Kalimantan and Sumatra) and Malaysia (Pahang, Sabah, and Sarawak). Cultivated in some areas of Borneo.	Unknown	Juicy, very acid fruit with turpentine and lemon taste: edible.
Mangifera	*Mangifera* Ding Hou	*M. casturi*	Polyembryonic	Indonesia	Endemic to South Kalimantan, Indonesia. Not known in the wild, only found in cultivation.	Unknown	Small fruits, with an attractive purple color and a distinctive aroma: edible.
Mangifera	*Mangifera* Ding Hou	*M. indica* cv. 'Kensington Pride'	Polyembryonic	Australia	First planted in Bowen, North Queensland, Australia. Pre-Australian origin is unknown. Grown throughout Australia.	40	Soft and juicy flesh with moderate to little fibre, sweet with a characteristic flavour excellent eating quality.
Mangifera	*Mangifera* Ding Hou	*M. indica* cv. 'Alphonso'	Monoembryonic	India	Prominently grown in India.	40	Firm to soft flesh, low in fibre content, sweet with characteristic aroma, very pleasant taste: edible.
Mangifera	*Mangifera* Ding Hou	*M. indica* cv. 'Tommy Atkins'	Monoembryonic	USA (Florida)	Originated and most widely grown commercial cultivar in USA.	40	Fruit with firm, medium juicy, medium amount of fibre of good eating quality. Highly resistant to anthracnose disease.

and the number INDELs, substitutions and SNPs present between the sequences were identified.

Nuclear gene sequence assembly

A set of single-copy nuclear genes was used to analyze phylogenetic relationships among the species. To analyze hybridization/introgression possibilities within the genus, it was necessary to develop individual gene trees to determine if they showed a discordance from the average phylogeny since different genes can have different evolutionary histories. The coalescence/ASTRAL approach, which employs multiple genes to develop gene trees to determine the degree to which they exhibit the same topology was applied.

Details of the genes were not available for *Mangifera* species. Therefore, *Citrus sinensis*, the closest relative of *M. indica* for which the details of single-copy nuclear genes were available[39] was used as the reference, to extract corresponding single-copy genes in mango. Single copy genes (107) in *C. sinensis* were mapped against the coding DNA sequences/gene models of *M. indica* cv. 'Alphonso'[29] in CLC-GWB. Out of 107, 47 were identified as single-copy genes in *M. indica* (Supplemental Table S3). Then, trimmed paired-end illumina reads of each species were mapped against 47 single-copy genes of *M. indica,* and consensus gene sequences were extracted. The same 47 gene sequences were extracted from the outgroup (*A. occidentale*) used in phylogenetic analysis.

Phylogenetic analysis

Chloroplast genome-based phylogenetic analysis

For chloroplast genomes-based phylogenetic analysis, sequences were imported to the Geneious and aligned using MAFFT alignment tool[30]. Two methods were used for phylogenetic analysis: Maximum likelihood (ML) method and Bayesian inference (BI) method. jMfodelTest v2.1.4[40] was used to select the best-fitting nucleotide substitution model (Supplemental Table S4). ML analysis was performed in RaXML GUI 2.0 (v 2.0.10)[41] with 1,000 bootstrap replicates. Bayesian analysis was carried out in Geneious software using MrBayes v. 3.2[42] (Supplemental Table S4). iTOL v.6 tool (https://itol.embl.de)[43] was used to visualize and edit the phylogenies. Using posterior probability (PP) and bootstrap support (BS) to evaluate the supports of the phylogenetic tree implemented under BI and ML methods respectively.

Nuclear gene-based phylogenetic analysis

For nuclear gene sequences, phylogenetic trees were generated using two approaches: gene concatenation and coalescent approach to analyze any topological incongruence and for a better understanding of evolutionary relationships among species.

Gene concatenation approach

All 47 single-copy genes were concatenated in the same order to obtain one long sequence per species. Sequences for all the species were imported to the Geneious 2022.2.2 software and aligned by MAFFT alignment. Phylogenetic trees were constructed using ML method and BI methods after selecting the best-fitting nucleotide substitution model by running jModelTest v2.1.4[40]. ML analysis was performed in RAxML (version 8)[41] with 1,000 bootstrap replicates, and Bayesian analysis was carried out in Geneious software using MrBayes v. 3.2[42] (Supplemental Table S4). iTOL v.6 tool (https://itol.embl.de)[43] was used to visualize and edit the phylogenetic trees.

Coalescent approach

Single ML gene trees were constructed using RAxML (version 8)[41]. The best-scoring ML tree was searched using a GTR + GAMMA model with 1000 bootstrap replicates. Low support branches (BS < 10%) in gene trees were collapsed to minimize the potential impacts of gene tree error for species tree reconstruction. The gene trees were used to construct a coalescent-based species tree using ASTRAL-III[44].

Results

Chloroplast genome assembly and annotation

Illumina sequencing conducted for the 19 samples belonging to 14 *Mangifera* species in this study resulted in 55,999,560 to 181,601,786 raw reads with 150 bp mean read length. The number of trimmed paired-end reads trimmed at 0.01 quality limits (Phred score > 20) ranged between 52,547,125 and 171,303,402 reads. As the data size of the trimmed reads of all 19 samples corresponded to over 20 x of the genome size, all were selected for the chloroplast assembly (Supplemental Table S1). Raw reads downloaded from NCBI for five *Mangifera* species (*M. odorata*, *M. sylvatica*, *M. persiciformis*, *M. hiemalis*, and *M. indica* cv. Tommy Atkins) had a total of 99,649,506 to 127,708,722 reads which ranged from 94,450,606 to 117,445,811 after trimming at 0.01 quality limits. For all the species, the mean coverage was higher than 20x genome size, which enabled them to be included in the analysis (Supplemental Table S2). The Get Organelle pipeline resulted in two output files for the chloroplast genome for each species/genotype, due to the possibility of the SSC occurring in both orientations in the chloroplast genomes in plants. Therefore, the two chloroplast sequences for each species were aligned with the reference (*M. indica*; Accession: NC_035239.1) in Clone Manager Professional 9 to select the sequence with the widely accepted SSC orientation (5'LSC3':5'IR13':5SSC3':3'IR25').

The size of the chloroplast genomes of 15 wild *Mangifera* species and three cultivars of *M. indica* ranged from 151,752 to 158,965 bp of which the smallest and the largest genomes were recorded for *M. caesia* and two *M. laurina* respectively (Table 2). The typical quadripartite structure of the chloroplast genome was recorded in all 16 *Mangifera* species and the lengths of LSC, SSC, and IR regions ranged between 86,507 to 98,334 bp, 18,319 to 19,064 bp, and 17,177 to 26,412 bp respectively where overall guanine–cytosine content (GC content) ranged from 37.6% to 37.9%. The chloroplast genomes for all species had the same number of total genes (115), rRNA (4) and tRNA (31) and protein encoding genes (80) (Table 2). Although the size of the chloroplast genomes varies across the *Mangifera* species, three cultivars of *M. indica*, two *M. laurina* samples, and two *M. altissima* samples had identical chloroplast genomes. Two *M. odorata* samples had slightly different chloroplast genome sizes, where the accession we sequenced had a genome size of 158,889 bp while the sample for which the data was downloaded from NCBI (*M. odorata**) had a genome size of 158,883 bp, representing a 6 bp difference. The length difference was due to two deletions revealed in *M. odorata**, one located in a non-coding region of LSC while the other located in the intron1 region of the *PetD* gene of LSC. The chloroplast genomes of two *M. pajang* samples collected from Treefarm, El Arish (*M. pajang*) and Durian Heaven Farm (*M. pajang*‡) had 57 bp difference in length with 27 variants (insertions,

Table 2. Annotation of the chloroplast genomes of *Mangifera* species.

Species/ genotype	Genome size (bp)	Overall GC content	LSC (bp)	SSC (bp)	IR (bp)	Total no. of genes	Total no. of protein coding genes	Total no. of tRNAs	Total no. of rRNAs
M. laurina	158,965	37.8%	87,714	18,427	26,412	115	80	31	4
M. laurina†	158,965	37.8%	87,714	18,427	26,412	115	80	31	4
M. pajang	158,830	37.8%	87,654	18,424	26,376	115	80	31	4
M. pajang‡	158,887	37.8%	87,709	18,426	26,376	115	80	31	4
M. caloneura	157,780	37.9%	86,672	18,350	26,379	115	80	31	4
M. odorata	158,889	37.8%	87,708	18,427	26,377	115	80	31	4
M. foetida	158,882	37.8%	87,706	18,422	26,377	115	80	31	4
M. altissima	157,780	37.9%	86,673	18,349	26,379	115	80	31	4
M. altissima†	157,780	37.9%	86,673	18,349	26,379	115	80	31	4
M. caesia	151,752	37.6%	98,334	19,064	17,177	115	80	31	4
M. zeylanica	157,604	37.9%	86,507	18,319	26,389	115	80	31	4
M. lalijiwa	157,779	37.9%	86,672	18,349	26,379	115	80	31	4
M. applanata	157,779	37.9%	86,672	18,349	26,379	115	80	31	4
M. casturi	158,942	37.8%	87,733	18,425	26,392	115	80	31	4
M. quadrifida†	158,889	37.8%	87,679	18,424	26,393	115	80	31	4
M. sylvatica†	158,025	37.9%	86,856	18,387	26,391	115	80	31	4
*M. sylvatica**	157,781	37.9%	86,712	18,347	26,361	115	80	31	4
*M. odorata**	158,883	37.8%	87,702	18,427	26,377	115	80	31	4
*M. persiciformis**	158,952	37.8%	87,566	18,536	26,368	115	80	31	4
*M. hiemalis**	158,838	37.8%	87,681	18,535	26,368	115	80	31	4
M. indica cv. 'Kensington Pride'	157,780	37.9%	86,673	18,349	26,379	115	80	31	4
M. indica cv. 'Alphonso'	157,780	37.9%	86,673	18,349	26,379	115	80	31	4
M. indica cv. 'Tommy Atkins'*	157,780	37.9%	86,673	18,349	26,379	115	80	31	4

GC, guanine or cytosine; IR, inverted repeats; LSC, large single copy; SSC, small single copy. * *Mangifera* species for which chloroplast genomes were assembled using raw data downloaded from NCBI. † *Mangifera* species collected from the Botanical Ark, Queensland, Australia. ‡ *Mangifera* species collected from the Durian Heavan Farm, Queensland, Australia. *M. pajang* is collected from TreeFarm, Queensland, Australia.

deletions, SNPs, substitutions) between two genomes. However, only four SNPs were identified within coding regions of *matK*, *atpA*, *rps2*, and *psbC* genes in LSC, and none of these SNPs resulted in a change in amino acid sequence causing no effect on the produced protein. Of the remaining SNPs, four were in non-coding regions of LSC, one in the intron region of the *pafI* gene and two in the intron region of the *NdhA* gene. Except for one insertion and one deletion observed in intron regions of *petD* and *trnK-UUU* genes, respectively, all other insertions, deletions, and substitution occurred in non-coding regions of both the LSC and SSC. In addition, except of one deletion, all identified insertions, were characterized as tandem repeats, spanning lengths of 1-2 bp. Upon examination of two *M. sylvatica* samples, it was noted that they had different chloroplast genome sizes. Specifically, the accession sequenced in the present study was 245 bp larger than the accession data downloaded from NCBI (*M. sylvatica**). Comparative analysis of annotated chloroplast genomes revealed 183 variants, including 119 SNPs, 29 insertions, 27 deletions, and eight substitutions in *M. sylvatica**. However, only 47 variants were in the coding region (46 SNPs, one insertion) resulting in codon change in 13 genes. The chloroplast sequence of *M. indica* cv. 'Kensington Pride' (Fig. 1) is a representation of the chloroplast sequence of the 16 *Mangifera* species, which have the same number of genes. Despite this consistency, differences exist in total chloroplast size, as well as the sizes of the LSC, IR1, and IR2, and the SSC regions.

Chloroplast phylogeny and identification of SNPs and INDELs

A multiple chloroplast sequence alignment conducted using *A. occidentale* as the outgroup followed by phylogenetic tree construction resulted in an ML tree and a Bayesian tree with same tree topology. BS and PP values of the final tree are presented in Fig. 2. The model of nucleotide substitutions for ML analysis was GTR + G, whereas, for the Bayesian analysis, it was TPM1uf + G. The tree developed with the ML approach showed a BS of 100 at most of the nodes and a PP of one in all the nodes. In the whole plastome tree, three main clades were identified. First, 16 *Mangifera* species were clustered into two distinct clades in which only *M. caesia* belonging to section *Dissidue* in the subgenus *Limus* was placed in the first clade (Clade A). The other 15 species were grouped into a separate clade indicating their evolutionary distinct relationship to *M. caesia,* which were then clustered into two subclades (Clade B and Clade C). Clade B included a total of eight species that belong to different categories in the classification. *M. pajang*, *M. foetida*, and *M. odorata* belong to the sub genus *Limus* while *M. casturi*, *M. quadrifida*, and *M. laurina* belong to the subgenus *Mangifera*. *M. persiciformis*, and *M. hiemalis* are two species placed under uncertain position in the classification. Within Clade B, species in subgenera *Mangifera* (Clade BI), *Limus* (Clade BII), and species being classified in an uncertain position (Clade BIII) have localized into well-supported distinct clades (BS = 100, PP = 1). The species belong to subgenera *Mangifera* and *Limus* and were sisters to each other and both together have become a sister clade to species placed in uncertain positions in the classification. Clade C had species belonging only to the subgenus *Mangifera*. Interestingly, four wild species (*M. lalijiwa*, *M. applanata, M. altissima*, and *M. caloneura*) were clustered with three cultivars of domesticated mango (*M. indica*) (Clade CI). Although species belonging to sections *Mangifera* and *Euantherae* are characterized by the presence of one and

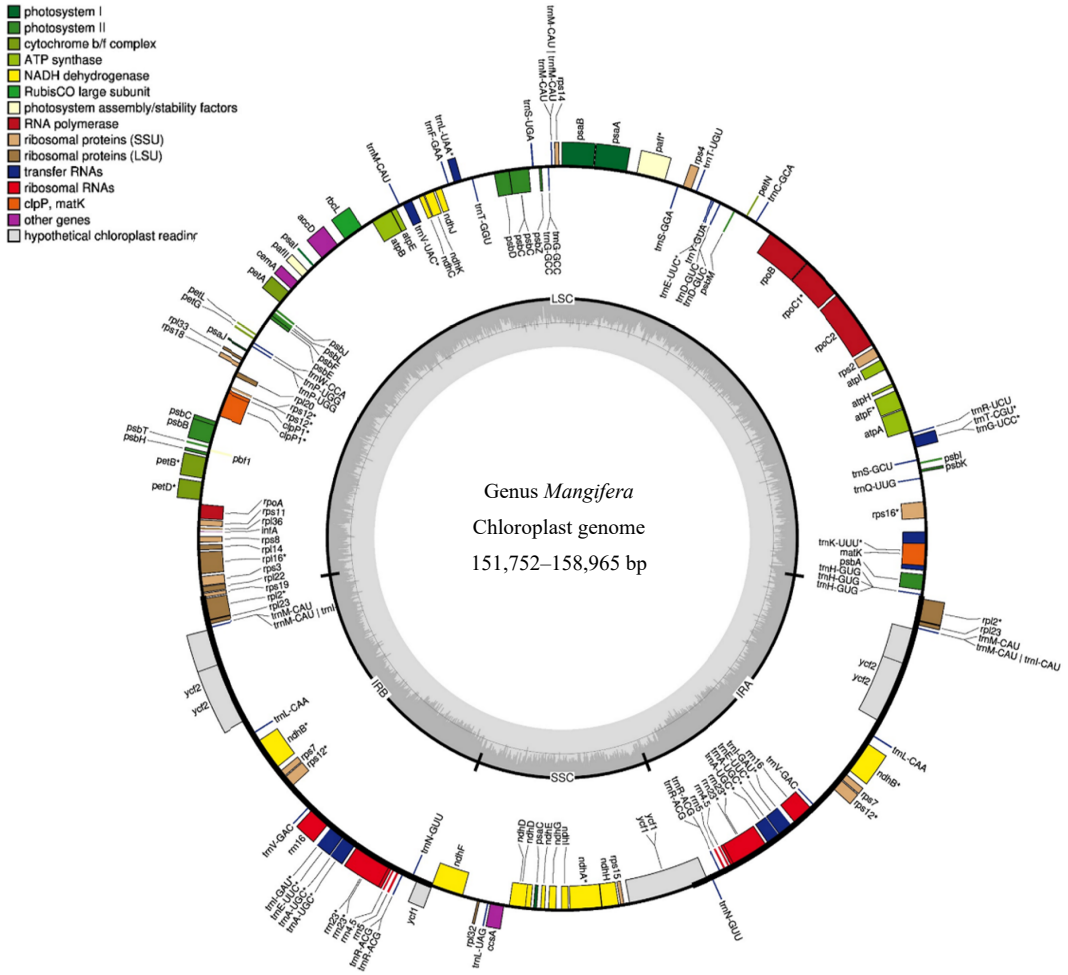

Fig. 1 Genome map of the chloroplasts in the genus *Mangifera*. The genome size of the 16 *Mangifera* species ranges from 151,752 to 158,965 bp for *M. caesia* and *M. laurina*, respectively. In the most outer circle, the thick black border/line indicates Inverted Repeat Regions (IR) whereas the thin lines indicate Large Single Copy (LSC) and the Small Single Copy (SSC). Genes inside the circle are transcribed in the clockwise direction whereas the genes outside the circle are transcribed in the counter-clockwise direction. Different colours are given for the genes with respect to their functions. The darker grey in the inner circle corresponds to GC content, whereas the lighter grey corresponds to AT content.

multiple fertile stamens respectively, *M. caloneura* in section *Euantherae* was clustered with species belonging to section *Mangifera*. Furthermore, *M. zeylanica*, and *M. sylvatica* (both samples); two species within Clade C were separately clustered into distinct clades (Clades CII and III). Therefore, the phylogeny based on whole chloroplast genome clustered species belong to different groups, inferring the close genetic and evolutionary relationships of their chloroplast genomes (Fig. 2).

The assembled chloroplast genomes were imported to Geneious software to conduct pairwise alignment to identify the number and types of variants present between the species clustered as sister taxa in the two main clades (Clade B and C) of the chloroplast phylogeny. In Clade B, two *M. laurina* samples had identical chloroplast genomes whereas two *M. odorata* samples had two structural variants (deletions). The chloroplast genome of *M. pajang* had a total of 27 structural variants including one substitution, 11 SNPs, seven insertions, and eight deletions compared to *M. pajang*. A total of 116 variants were observed between *M. laurina* and *M. casturi* while 119 variants were found between *M. casturi* and *M. quadrifida*. Furthermore, a total of 75 variants were found between *M. odorata* and *M. pajang* while there were 108 variants between *M. odorata* and *M. foetida* (Table 3). In addition, the two species *M. persiciformis* and *M. hiemalis,* for which the chloroplast genomes were assembled from raw read data available in NCBI, differed by 45 variants, overall revealing the close evolutionary relationships among the taxa in Clade B. In Clade CI, pairwise comparison of wild species with *M. indica* cv Kensington Pride showed that, *M. altissima* and *M. indica* had identical chloroplast sequences. Moreover, *M. lalijiwa* and *M. applanata* also had an identical chloroplast genome which differed from *M. indica* only by having a single nucleotide deletion located in a non-coding region in LSC. Furthermore, despite reporting distinct morphological characteristics from *M. indica*, *M. caloneura* only had one single nucleotide insertion and one single nucleotide deletion in non-coding regions compared to *M. indica*. Diversity within the chloroplast genomes clustered in Clade CI was very low.

Nuclear gene phylogeny

Concatenation-based nuclear phylogeny

The same approach was used to construct a nuclear phylogeny with a concatenation-based approach as was

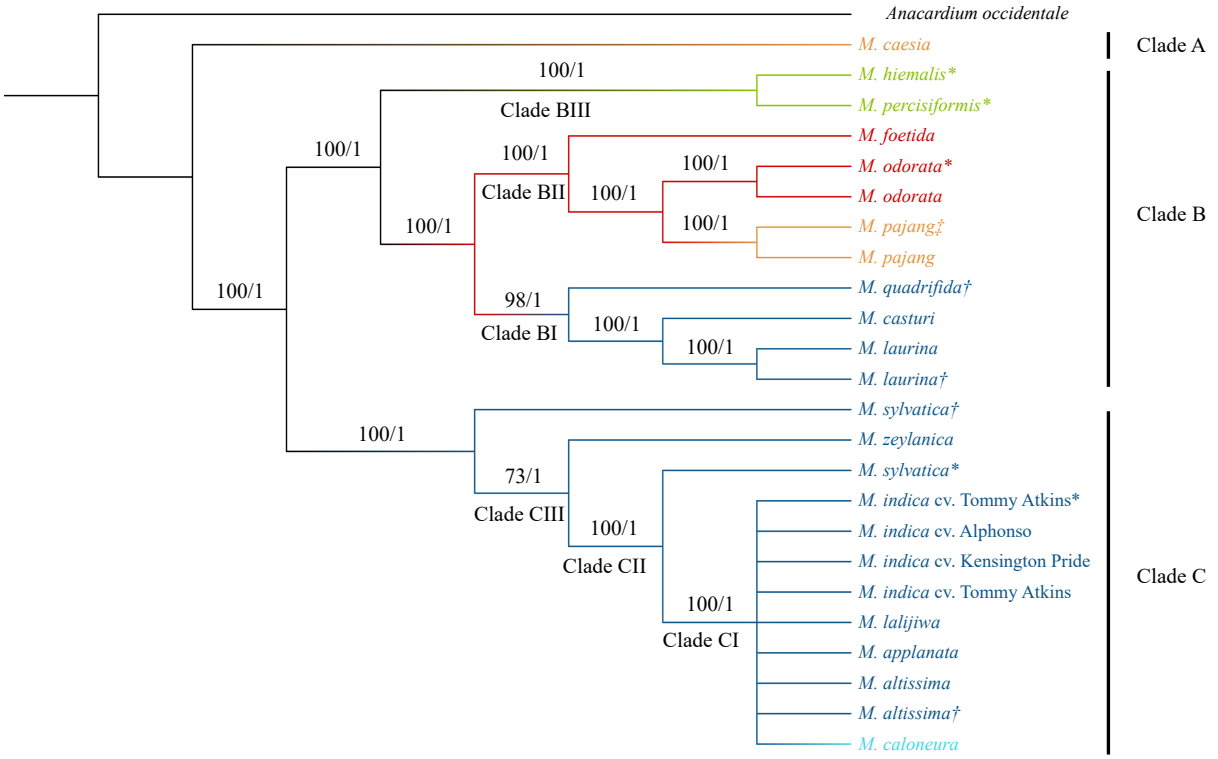

Fig. 2 Phylogenetic tree developed for *Mangifera* species based on whole chloroplast genomes. The phylogenetic tree of 24 accessions belongs to 16 species with *A. occidentale* used as the outgroup. Trees were generated using Maximum Likelihood (ML) and Bayesian inference (BI) method. Numbers associated with the branches are ML bootstrap value (/100) and BI posterior probabilities (/1). Dark Blue: Sub genus *Mangifera*, Section *Mangifera*, Light blue: Sub genus *Mangifera*, Section *Euantherae,* Red: Sub genus: *Limus*, Section *Perrennis,* Yellow: Sub genus: *Limus*, Section: *Deciduae*, Light Green: species placed in uncertain position in the classification. * *Mangifera* species for which chloroplast genomes were assembled using raw data downloaded from NCBI. † *Mangifera* species collected from the Botanical Ark, Queensland, Australia. ‡ *Mangifera* species collected from the Durian Heaven Farm, Queensland, Australia.

applied in constructing the chloroplast phylogeny. *A. occidentale* was used as the outgroup. A total of 47 common single-copy nuclear genes out of 107[39] were identified and selected for *Mangifera* species. The multiple-sequence alignment was 71,881 bp in length and ML and Bayesian trees resulted in almost the same tree topology. The final tree with BS values and PP values is presented in Fig. 3a. Although some of the nodes showed less BS support values, all the nodes were supported with high PP values. The model of nucleotide substitutions for ML analysis was GTR + I + G whereas TPM1 + I + G was used for the Bayesian analysis.

Except for *M. sylvatica* and *M. quadrifida,* the other eight species belonging to subgenus *Mangifera* were clustered into one main distinct clade. Among the eight clustered species, two *M. laurina* samples clustered as sister taxa and is the most distinct clade from the others. Of the seven remaining species, *M. lalijiwa*, *M. applanata* with *M. casturi* were clustered into one clade while *M. altissima*, *M. caloneura*, *M. zeylanica*, and the *M. indica* cultivars were clustered into another clade within the main clade. Two *M. altissima* samples were clustered into one clade and *M. casturi* and *M. lalijiwa* were sister taxa to each other. Furthermore, showing the close genetic relationship of the two *M. indica* cultivars (Kensington Pride and Tommy Atkins) to *M. zeylanica* and *M. altissima* revealed a close evolutionary relationship of the two wild species to domesticated mango. Moreover, although two *M. sylvatica* species closely related to species in the domesticated clades in chloroplast phylogeny, they were clustered with the two species placed in an uncertain position in the classification in the nuclear phylogeny. In addition, *M. quadrifida* was clustered with *M. foetida* and *M. pajang*; two species belong to the subgenus *Limus*. Both chloroplast and concatenation-based nuclear phylogenies revealed that *M. caesia* is evolutionarily distant from the rest of the *Mangifera* species (Figs 2 & 3a). Grouping of species in both chloroplast genome and nuclear genes-based analysis does not completely concur with the accepted classification[6] for genus *Mangifera.* Incongruence in tree topologies could be seen between the phylogenies developed based on the whole plastome genome and the nuclear genes.

Coalescence-based nuclear phylogeny

Previously proposed hybrids and their proposed parents were included in the dataset and the possibility of hybridization events also were observed for some species when compared chloroplast and concatenation-based nuclear phylogenies. Therefore, to further analyze the phylogenetic relationships among *Mangifera* species with respect to nuclear genes, a coalescence approach was utilised to develop individual nuclear gene trees thereby developing a species tree. Individual gene trees were analyzed to see close evolutionary relationships among species.

In coalescence-based species tree, local posterior probability support (LPP) values are indicated in the branches (/1). In both concatenation and coalescence based nuclear phylogenies, except *M. quadrifida*, species belonging to subgenus *Mangifera,*

Table 3. INDELs, SNPs and substitutions identified with respect to clustering pattern in chloroplast phylogeny.

Clade name in the phylogenetic tree	Species/ genotypes in comparison	Species/ genotype A	Species/ genotype B	Total no. of variations between A vs B	Types of variation			
					Insertions	Deletions	SNPs	Substitutions
BI	*M. laurina* *M. laurina*†	*M. laurina*	*M. laurina*†	0	–	–	–	–
	M. casturi *M. quadrifida*†	*M. laurina*	*M. casturi*	116	30	30	51	5
		M. casturi	*M. quadrifida*	191	32	25	119	15
BII	*M.odorata* *M. odorata**	*M. odorata*	*M. odorata**	2	–	2	–	–
	M. pajang *M. pajang*‡	*M. pajang*	*M. pajang*‡	27	7	8	11	1
		M. odorata	*M. pajang*	75	17	16	39	3
	M. foetida†	*M. odorata*	*M. foetida*	108	21	16	68	3
BIII	*M. persiciformis** *M. hiemalis**	*M. persiciformis**	*M. hiemalis**	45	9	9	27	–
CI	*M. altissima* *M. altissima*†	*M. indica* cv. 'Tommy Atkins'	*M. indica* cv. 'Kensington Pride'	0	–	–	–	–
	M. lalijiwa *M. applanata* *M. caloneura* *M. indica* cv. 'Kensington Pride' *M. indica* cv. 'Tommy Atkins' *M. indica* cv. 'Alphonso'	*M. indica* cv. 'Tommy Atkins'	*M. indica* cv. 'Tommy Atkins'*	0	–	–	–	–
		M. indica cv. 'Alphonso'	*M. indica* cv. 'Kensington Pride'	0	–	–	–	–
		M. indica cv. 'Alphonso'	*M. indica* cv. 'Tommy Atkins'	0	–	–	–	–
		M. altissima	*M. altissima*†	0	–	–	–	–
		M. altissima	*M. indica* cv. 'Kensington Pride'	0	–	–	–	–
		M. lalijiwa	*M. indica* cv. 'Kensington Pride'	1	–	1	–	–
		M. applanata	*M. indica* cv. 'Kensington Pride'	1	–	1	–	–
		M. caloneura	*M. indica* cv. 'Kensington Pride'	2	1	1		
		M. lalijiwa	*M. applanata*	0	–	–	–	–
		M. caloneura (Xoài Quéo)	*M. lalijiwa*	3	2	1	–	–
CII	*M. sylvatica** *M. indica cv.* 'Kensington Pride'	*M. sylvatica**	*M. indica* cv. 'Kensington Pride'	96	22	14	57	3
CIII	*M. zeylanica* *M. indica cv.* 'Kensington Pride'	*M. zeylanica*	*M. indica* cv. 'Kensington Pride'	175	18	26	122	9

* Species for which chloroplast genomes were assembled using raw data downloaded from NCBI. † *Mangifera* species collected from the Botanical Ark, Queensland, Australia. ‡ *Mangifera* species collected from the Durian Heavan Farm, Queensland, Australia.

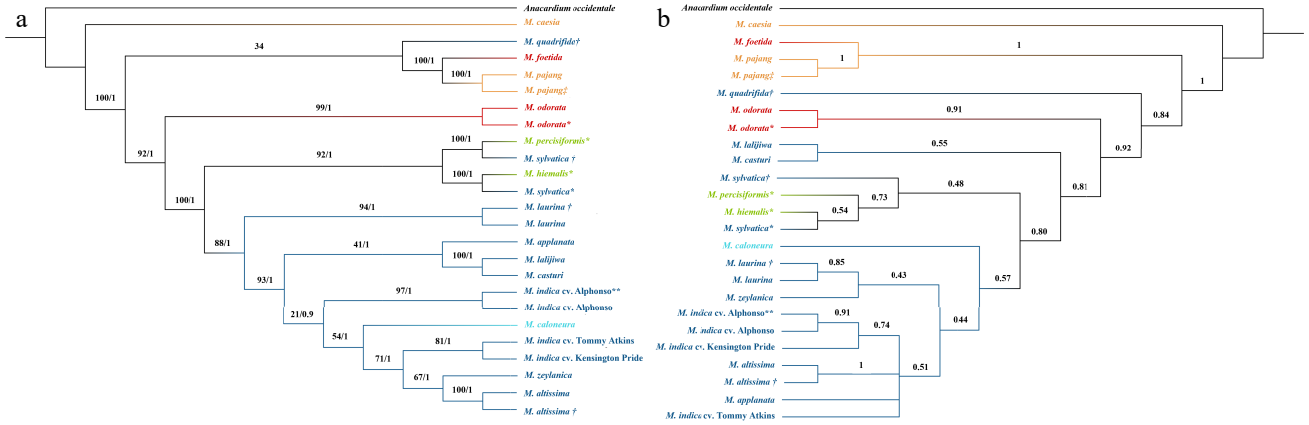

Fig. 3 Phylogenetic tree developed for *Mangifera* species based on a selected set of nuclear genes using (a) concatenation and (b) coalescence-based methods. Phylogenetic tree of 24 accessions with *A. occidentale* used as the outgroup. Concatenation-based trees were generated using Maximum Likelihood (ML) and Bayesian inference (BI) methods and consensus tree is shown in the figure. Numbers associated with the branches are ML bootstrap value (/100) and BI posterior probabilities (/1). In the coalescence-based tree (ASTRAL tree), numbers associated with branches are local posterior probability values (/1). Dark Blue: Sub genus *Mangifera*, Section *Mangifera*, Light blue: Sub genus *Mangifera*, Section *Euantherae*, Red: Sub genus: *Limus*, Section *Perrennis*, Yellow: sub genus*: Limus*, Section: *Deciduae*. * Species for which nuclear genes were extracted using raw data downloaded from NCBI. ** *M. indica* cultivar from which gene models were downloaded from NCBI and used to create local database in CLC-GWB for the selection of single copy nuclear genes in *M. indica*. † *Mangifera* species collected from the Botanical Ark, Queensland, Australia. ‡ *Mangifera* species collected from the Durian Heaven Farm, Queensland, Australia.

and species placed in uncertain positions in the classification were clustered in one clade with high support values (BS = 100, PP = 1, LPP = 0.81) (Fig. 3). Within this clade, the pattern of clustering into sub-clades was different for some species between the two nuclear phylogenies but BS and LPP support values also were low for some sub-clades. In both concatenation and coalescence-based phylogenies, *M. casturi* and *M. lalijiwa* are clustered together as sister taxa (BS = 100, PP1, LPP = 0.55) and *M. hiemalis*, *M. persiciformis*, and two *M. sylvatica* samples were clustered into one sub-clade (BS = 100, PP = 1, LPP = 0.48). In the coalescence-based tree, *M. hiemalis*, *M. sylvatica*, and *M. persiciformis* were closely related to six species belonging to the subgenus *Mangifera* (*M. altissima*, *M. applanata*, *M. indica*, *M. caloneura*, *M. zeylanica*, and *M. laurina*). However, in the concatenation-based tree, *M. casturi*, and *M. lalijiwa* were more closely related to the six species than *M. sylvatica*, *M. persiciformis*, and *M. hiemalis*.

M. odorata is a proposed hybrid between *M. indica* and *M. foetida*, and *M. casturi* is a proposed hybrid between *M. indica*, and *M. quadrifida*. Within the present dataset, there are both parental and hybrid species. Therefore, 47 nuclear gene trees were analyzed to support the hybridity by recording the number of gene trees where the hybrids were clustered with their parents. Out of 47 gene trees, *M. odorata* was clustered with *M. indica* as sister taxa in only four gene trees and they were not supported with high BS values (Table 4, Supplemental Fig. S1). Similarly, *M. odorata* was clustered with *M. foetida* as sister taxa only in four gene trees and one of the trees showed less BS value (Table 4, Supplemental Fig. S1). *M. casturi,* was clustered with *M. indica* as a sister taxa in only two gene trees where BS values were weak in one of them (Table 4, Supplemental Fig. S1). Furthermore, only one gene tree had *M. casturi* and *M. quadrifida* clustered into one clade that had less BS. Therefore, for both the proposed hybrid species, the number of gene trees in which the proposed hybrids clustered with their parental species was low and showed low BS values.

According to nuclear gene phylogeny, close evolutionary relationship between *M. zeylanica* and *M. indica* was observed where *M. zeylanica* was clustered with the species in domesticated clade. Analyzing individual gene trees revealed that *M. zeylanica* was clustered with *M. indica* as sister taxa in 11 gene trees and with *M. altissima* in eight gene trees while in some of the other gene trees, *M. zeylanic*a clustered with species in domesticated clade. In addition, it was also observed that there is a close evolutionary relationship between *M. hiemalis, M. persiciformis*, and *M. sylvatica* in nuclear phylogenies. *M. sylvatica* species were clustered with *M. hiemalis* as sister taxa in 12 gene trees and with *M. persiciformis* in nine gene trees. Furthermore, all three species (*M. sylvatica*, *M. persiciformis*, and *M. hiemalis*) were clustered into one subclade in four gene trees. However, individual gene trees for *M. zeylanica*, and *M. sylvatica* showed less BS support when clustering with *M. indica* and *M. hiemalis/M. persiciformis* respectively (Table 4, Supplemental Fig. S1). Therefore, it was assumed that *M. zeylanica* might have undergone domestication and there is also a possibility that *M. sylvatica* may have cross-hybridised with *M. hiemalis* or *M. persiciformis* during the evolution of these species.

Discussion

Determination of phylogenetic relationships among crop species provides basic information for predicting their evolutionary history, taxonomical classification, and evaluating their diversity and importance in plant breeding[45]. Although genetic analysis of plants has improved rapidly with advanced sequencing technology, many phylogenetic studies in the genus *Mangifera* have relied on a set of molecular markers such as amplified fragment length polymorphisms (AFLP), rapid amplified polymorphic DNA and simple sequence repeats and the sequencing of limited numbers of targeted regions in the chloroplast genome and nuclear ribosomal DNA[10,13–18].

Chloroplast genomes for seven species were assembled for the first time in this study for *M. pajang*, *M. altissima*, *M. caesia*, *M. lalijiwa*, *M. zeylanica*, *M. appalanta*, and *M. casturi*. Different pipelines and programs are available to assemble organelle genomes. Here, CAP and Get Organelle pipeline have been used. The two approaches used in CAP (reference-guided mapping and *de-novo* assembly) eliminate many errors in genomes developed from each approach giving a highly accurate final chloroplast genome. The Get Organelle pipeline is also capable of generating all possible arrangements of the chloroplast genome present[36]. Therefore, a comparison of chloroplast genomes generated from CAP and the Get Organelle pipeline validated the development of highly accurate final chloroplast genomes for all the species. More genes have been annotated in our analysis compared to previous studies, which reported a total of 112 genes (78 protein-coding genes, 30 tRNA genes, four rRNA genes)[46] and 113 genes (79 protein-coding genes, 30 tRNA genes, four rRNA genes)[19].

Phylogenetic relationships within the genus *Mangifera* showed topological incongruence for some species with respect to whole chloroplast and nuclear genes trees which maybe caused by introgressive hybridization, allopolyploidy or incomplete lineage sorting. Reproductive compatibility between different species allows the native cytoplasm of a species to be easily replaced by another through hybridization which has been detected both in animals (mitochondrial capture)[47] and plants (chloroplast capture)[48]. In plants, chloroplast capture events have been reported in many plant families[49–52]. Hybridization followed by recurrent backcrossing have explained discrepancies between chloroplast and nuclear gene-based phylogenies in diverse families of plants[53–56]. In mango, evidence for inter-specific reproductive compatibility was reported for *M. indica* and *M. laurina*. A cross between *M. indica*, and *M. laurina* have produced 60 successful hybrids[57]. Hybrid origins were reported for *M. odorata* and *M. casturi*.

Close genetic relationship between *M. applanata*, and *M. altissima has* been reported in a phylogenetic analysis conducted based on *Maturase K* gene[15]. In the present study, *M. laijiwa*, *M. applanata*, *M. altissima*, and *M. caloneura* were clustered with *M. indica* in the chloroplast phylogeny sharing 99.9% sequence similarity. These four wild relatives clustered with domesticated mango into a distinct clade even in concatenation-based nuclear phylogeny showing their close evolutionary relationships whereas only *M. laijiwa* out of the above four species clustered separately in the coalescent approach. Furthermore, *M. indica* cultivars Kensington Pride and Tommy Atkins were more closely related to *M. altissima* than *M. indica* cv. 'Alphonso' in concatenation-based nuclear phylogeny failing to resolve *M. indica* from *M. altissima*. A close evolutionary relationship between *M. altissima*, and *M. indica* was also confirmed in the coalescence approach. Due to remarkably

Table 4. Gene trees indicating clustering of suggested hybrids/ wild relatives with their proposed parents as sister taxa.

Gene name	Gene tree no.	Species proposed to be hybrid				Species supposed to be undergone domestication/hybridization		Trees in which *M. sylvatica* clusters with *M. persiciformis* in the same clade as sister taxa
		M. odorata		*M. casturi*		*M. zeylanica*	*M. sylvatica*	
		Trees in which *M. odorata* clusters with *M. indica* in the same clade as sister taxa	Trees in which *M. odorata* clusters with *M. foetida* in the same clade as sister taxa	Trees in which *M. casturi* clusters with *M. indica* in the same clade as sister taxa	Trees in which *M. casturi* clusters with *M. quadrifida* in the same clade as sister taxa	Trees in which *M. zeylanica* clusters with *M. indica* in the same clade as sister taxa	Trees in which *M. sylvatica* clusters with *M. hiemalis* in the same clade as sister taxa	
4-alpha-glucanotransferase	1	×	×	×	×	√	×	×
A49-like RNA polymerase I associated factor	2	×	×	×	×	√	×	×
Acyl-CoA dehydrogenase, C-terminal domain	3	×	×	×	×	×	√	×
Alphabeta hydrolase family	4	√	×	×	×	√	×	√
Aminomethyltransferase folate-binding domain	5	×	×	×	×	×	×	×
Aminopeptidase I zinc metalloprotease (M18)	6	×	×	×	×	×	×	×
ArgJ family	7	×	×	×	×	×	×	×
Armadillobeta-catenin-like repeat	8	×	×	×	×	×	×	×
Brix domain	9	×	×	×	×	×	×	√
Cactus-binding C-terminus of cactin protein	10	×	×	×	×	×	×	×
Carbon-nitrogen hydrolase	11	√	×	×	×	×	×	×
CobWHypBUreG, nucleotide-binding domain	12	×	×	×	×	×	×	×
Cohesin loading factor	13	×	×	×	×	×	×	×
Creatinase Prolidase N-terminal domain	14	×	×	×	×	×	×	×
Cyclophilin type peptidyl-prolyl *cis*-trans isomeraseCLD	15	×	×	×	×	×	×	×
Cytochrome b5-like HemeSteroid binding domain	16	×	×	×	×	×	×	×
DDRGK domain	17	×	√	×	×	×	×	×
Dienelactone hydrolase family	18	×	√	×	×	×	√	×
Divergent CRALTRIO domain	19	×	×	×	×	×	√	×
Dual specificity phosphatase, catalytic domain	20	×	×	×	×	√	√	×
Dynamin family (2)	21	×	×	×	×	×	×	×
ER membrane protein complex subunit 1, C-terminal	22	×	×	×	×	×	×	×
Eukaryotic protein of unknown function (DUF866)	23	×	×	√	×	×	×	×
FAD binding domain	24	×	×	×	×	√	×	×
Glucose-6-phosphate isomerase	25	×	×	×	×	×	×	√
Glucosidase II beta subunit-like protein	26	×	×	×	×	×	√	×

(to be continued)

Table 4. (continued)

Gene name	Gene tree no.	Species proposed to be hybrid				Species supposed to be undergone domestication/hybridization		Trees in which *M. sylvatica* clusters with *M. persiciformis* in the same clade as sister taxa
		M. odorata		*M. casturi*		*M. zeylanica*	*M. sylvatica*	
		Trees in which *M. odorata* clusters with *M. indica* in the same clade as sister taxa	Trees in which *M. odorata* clusters with *M. foetida* in the same clade as sister taxa	Trees in which *M. casturi* clusters with *M. indica* in the same clade as sister taxa	Trees in which *M. casturi* clusters with *M. quadrifida* in the same clade as sister taxa	Trees in which *M. zeylanica* clusters with *M. indica* in the same clade as sister taxa	Trees in which *M. sylvatica* clusters with *M. hiemalis* in the same clade as sister taxa	
Glycosyl hydrolases family 31	27	×	×	×	×	×	√	×
Glyoxalase bleomycin resistance protein dioxygenase superfamily	28	×	×	×	×	×	×	×
Hydroxyacylglutathione hydrolase C-terminus	29	×	×	×	×	×	×	×
Methyltransferase domain	30	×	√	×	×	×	×	×
NmrA-like family	31	×	×	×	×	√	√	×
N-terminal domain of lipoyl synthase of Radical_SAM family	32	×	×	×	×	×	×	×
PAP2 superfamily C-terminal	33	×	×	×	×	×	×	×
Prolyl oligopeptidase, N-terminal beta-propeller domain	34	×	×	×	×	×	×	×
Putative tRNA binding domain	35	×	×	×	×	√	√	×
Pyruvate phosphate dikinase, AMPATP-binding domain	36	×	×	×	×	×	√	√
Redoxin	37	√	×	×	×	×	×	×
Ribosomal protein L13	38	×	×	×	√	√	√	×
RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	39	×	×	×	×	×	√	×
Rubrerythrin	40	×	×	×	×	×	×	√
snoRNA binding domain, fibrillarin	41	×	×	×	×	√	×	×
Sodium Bile acid symporter family	42	×	√	×	×	√	×	×
TFIIS helical bundle-like domain	43	×	×	×	×	√	×	×
Transcription factor TFIIB repeat	44	√	×	×	×	×	√	√
tRNA synthetase class II core domain (G, H, P, S and T)	45	×	×	×	×	×	×	√
Uncharacterized ACR, YdiUUPF0061 family	46	×	×	√	×	×	×	√
WLM domain	47	×	×	×	×	×	×	√

close evolutionary relationships observed in chloroplast and nuclear phylogenies, we suggest these four wild relatives and cultivated mango are very closely related and might have shared or descended from a common ancestor.

Although a single domestication event has been reported for *M. indica* based on historical records[2], two independent domestication events have been proposed in India and Indochina[7]. A population genomics study[58] suggested that mango domestication is a complex process and it may involve multiple domestication events and interspecific hybridization; two common phenomena observed in the domestication of perennial fruit crops. Their results indicated a high genetic diversity among *M. indica* cultivars distributed outside of the region where the mango originated and a unique genetic diversity in Southeast Asian cultivars compared to other populations. Furthermore, they suggest that the origin and initial cultivation of mango may have taken place in Southeast Asia and further improvement and domestication may have occurred in India. In addition, cross-hybridization was highly likely to occur between wild relatives and *M. indica* at the early stages of domestication due to the presence of a high number of species which is supported by evidence for crossbreeding. Thus, apart from descending from a common ancestor, cross-hybridization between *M. indica* and the four wild relatives is also a possible phenomenon that may have further contributed to the close evolutionary relationships observed in our study. However, this could be further supported by including multiple replicates for the species which is a limitation in this study.

M. zeylanica, is an endemic species to Sri Lanka. A close evolutionary relationship was observed in the concatenation-based nuclear phylogeny between *M. zeylanica*, and *M. indica* despite having a distinct chloroplast genome. Therefore, it was hypothesized that cross-hybridization might have occurred between an early lineage of *M. zeylanica*, and *M. indica* or its close wild relative. Since the species have a distinct chloroplast genome, it was assumed that *M. indica* may have most likely acted as the paternal parent, resulting in hybrids that carry the chloroplast genome of *M. zeylanica* and nuclear genes of both *M. zeylanica*, and *M. indica* or its close relative. The nuclear phylogeny/species tree based on the coalescence approach also showed a close relationship between *M. indica*, and *M. zeylanica*. Clustering of *M. zeylanica* with *M. indica* and with *M. altissima* which is a close relative to *M. indica* suggested the possibility of *M. zeylanica* having a hybrid origin. But as the BS/PP and LPP values are relatively low for this clade in gene trees as well as in both the consensus trees, it is also possible that the set of genes is not sufficiently variable to give a better resolution in the phylogeny. Therefore, it is difficult to conclude the cross-hybridization of *M. zeylanica*.

M. casturi is a cultivated species in Indonesia. This endemic species is only found in cultivation[59] and was proposed to be a natural hybrid between *M. indica*, and *M. quadrifida* according to a SNP analysis[25]. Since *M. casturi* has shown a higher affinity to *M. indica* than to *M. quadrifida* instead of being direct intermediate between two species, it was further suggested that *M. casturi* is most likely a result of an F1 hybrid backcrossed with *M. indica*[25]. Microsatellite marker-based analysis showed broad genetic variation among four *M. casturi* samples and DNA barcoding-based phylogenetic analysis suggested several species as ancestors for *M. casturi*[24]. Genetic variation has also been confirmed between 16 accessions of *M. casturi* using SNP markers (N. Dillon, pers. comm.). Therefore a combination of microsatellite and DNA barcoding data support that *M. quadrifida* and *M. indica* have hybridised to result in M. *casturi* and F1 hybrids may have further hybridized with the ancestors of the parental species or multiple other *Mangifera* species to generate hybrids with high genetic diversity[24]. In the present study, *M. casturi* has a distinct chloroplast genome and it is closely related to *M. quadrifida* in chloroplast phylogeny. However, a close evolutionary relationship was observed between *M. casturi* and species in the domesticated clade in the concatenation-based nuclear phylogeny where it clusters with *M. lalijiwa* as sister taxa and distinctly related to *M. quadrifida*. In contrast, in the coalescence approach, *M. casturi* showed a relatively distant evolutionary relationship with *M. indica*, and *M. quadrifida* both in species trees and in individual gene trees. Therefore, according to our results, coalescence-based nuclear phylogeny and gene trees don't strongly support the parentage of *M. indica*, and *M. quadrifida* for *M. casturi*. Since a very low number of genes are shared between *M. indica/M. quadrifida*, and *M. casturi*, is not possible that *M. casturi* is a first-generation hybrid if the two species are the parents. The F1 of *M. casturi* may have cross-hybridized with other wild relatives as previous study suggested[24]. Also, the absence of replicates for *M. casturi*, and *M. quadrifida* and other wild relatives limit analysis of any other species for the hybrid origin of *M. casturi*.

M. laurina is a cultivated species in Indonesia where its wild distribution ranges from Myanmar, Cambodia, Vietnam and Malesia, Thailand to New Guinea. Analysis of *ITS* genomic region[18] have revealed close evolutionary relationship between *M. laurina*, and *M. indica*. Analysis of *Maturase K* chloroplast genomic region has differentiated Indonesia and Thailand specimens collected for *M. laurina*. Since common interspecific hybridization has been suggested for this species[14], it is possible that *M. laurina* may have cross-hybridized with other species after introduction to the regions where it is widely cultivated. Due to the relatively close evolutionary relationship observed between *M. laurina*, and *M. indica* in nuclear gene analysis despite the chloroplast genome being distinct, it might be possible to occur hybridization between the early lineage of *M. laurina* and *M. indica* its close relative. Current data and results only support the close evolutionary relationship between the two species, but further analysis should be conducted with multiple samples for both species.

Among *M. pajang*, *M. foetida*, *M. odorata*, *M. persiciformis*, and *M. hiemalis* clustered within the same main clade in chloroplast phylogeny, *M. pajang* is an endemic species originating from and cultivated in Borneo, Indonesia. Based on the AFLP marker analysis, *M. odorata* is proposed as a hybrid between *M. indica*, and *M. foetida* and it has shown more affinity to *M. foetida* than to *M. indica*[18,23]. The present results also confirm that *M. odorata* is closely related to *M. foetida* than to *M. indica* according to both chloroplast and nuclear phylogenies. Since chloroplast genomes are relatively conserved, have less rate of evolution and in general, shows maternal inheritance (in angiosperms), hybrids share the chloroplast genomes of maternal parents. A study conducted on the inheritance of *Solanum* chloroplast genomes in four known interspecific hybrids revealed that two hybrids had identical chloroplast genomes while the other two showed only 2 bp difference with respect to their maternal parents[31,60]. Furthermore, it was also revealed that only one hybrid had two substitutions in the coding sequence and in

intergenic region while the other three were consistent with the maternal parent. In the present study, although the chloroplast genomes of *M. foetida* and *M. odorata* differ in 7 bp, there are 108 variants between the two species including 27 SNPs and nine insertions. In both concatenation and coalescence approaches for nuclear genes, *M. odorata* showed a relatively distant evolutionary relationship with *M. indica*. Individual gene trees clustered the *M. odorata* with each proposed parent in four gene trees only and some clades showed weak BS. Although the whole chloroplast genome and multiple nuclear genes provide more information compared to molecular markers, evidence for the hybrid origin of *M. odorata* is not strong enough and the parentage of *M. odorata* is inconclusive according to the results. Therefore, further analysis is required with populations for proposed parents and the hybrid to confirm the hybridity.

Another discrepancy observed from the chloroplast and nuclear trees is related to the position of *M. sylvatica*. Previous studies have revealed a close evolutionary relationship between *M. indica* and *M. sylvatica* based on restriction fragment length polymorphism (RFLP)[10], *ITS*[18] marker analysis and whole chloroplast genome analysis[19]. Here, two *M. sylvatica* samples had different chloroplast genome sizes and had some structural differences. Therefore, *M. sylvatica* samples didn't cluster as sister taxa in chloroplast phylogeny. Another study[61] also reported that the *M. sylvatica* chloroplast genome assembled has a different length (157,368 bp) compared to the one available in NCBI and clustering them into two separate subclades. Since two *M. sylvatica* samples have been collected from different countries, the suggestion is that the regional separation might have mediated these evolutionary differences. Furthermore, another assembled *M. sylvatica* chloroplast genome is 158,063 bp in size[46]. Therefore, different chloroplast genome sizes and their structural variations of *M. sylvatica* might have occurred due to their different geographical distribution. Despite having structural variations, two *M. sylvatica* samples in our study were also closely related to *M. indica*. However, in nuclear phylogenies, they were nested with *M. hiemalis* and *M. persiciformis* suggesting that *M. sylvatica* might have a hybrid origin which has been occurred a long time ago, but the low BS values in individual gene trees do not provide strong support for this hypothesis.

Topological incongruence observed by the chloroplast genome and single-copy nuclear gene-based phylogenies reveal that there is a potential for inter-specific hybridization in the genus. But less BS values and weak resolution in gene trees of coalescence approach and low BS/PP/LPP support values in some of the branches of concatenation-based nuclear phylogeny and species tree are clear evidence that the nuclear genes are not well distinguished/ might not vary across the group of species studied. Less variability of nuclear genes and absence of multiple replicates for proposed hybrids limited conclusions about possible hybridization event/s and hybrid origin of *M. odorata*, and *M. casturi*. Although both the proposed parents are present, phylogenies will show their close evolutionary relationships if it is a recent generation hybrid. Therefore, the results of this study suggests that the whole group is sufficiently closely related with each other, so we needed a large amount of data to get well-resolved and highly supported phylogenetic trees. The history of evolution of the species and hybridization is complex in the genus and requires more species to get a better understanding. However, it is possible that out of 69 distinct species identified in the genus, some or many of them may have either domestication input or cross-hybridized with other wild relatives.

Conclusions

The analysis of determining evolutionary relationships within the genus *Mangifera* revealed a close genetic relationship among species and discrepancies between whole plastome and nuclear gene-based phylogenies. We suggest that the five species including *M. indica*, *M. altissima*, *M. applanata*, *M. caloneura*, and *M. lalijiwa* are very closely related and might have descended from the same common ancestor. It was difficult to validate the hybrid origin of *M. odorata*, and *M. casturi* as suggested previously due to the absence of multiple replicates for the proposed parents within our dataset, clustering of the proposed parent in only a few number of gene trees, and due to weak support obtained in gene trees. Relatively high numbers of gene trees showed a close evolutionary relationship between *M. zeylanica*, and *M. indica,* and *M. sylvatica* and *M. hiemalis/M. persiciformis*. However, the evidence did not strongly support the possible hybridization due to weak BS/PP and LPP supports. Moreover, it was observed that geographical proximity might have facilitated possible hybridization events. Despite limited number of species used in the study, it seems that evolution and hybridization in the genus *Mangifera* is a complex process. This is the first comparative analysis of evolutionary relationships within the genus with whole chloroplast genome and multiple nuclear genes. These findings provide an understanding about the nature of hybridization within the genus between wild and domesticated mangoes revealing potential domestication input into some species. Validation of hybridity and accuracy of evolutionary relationships within the genus can be highly supported and improved by adding more species including multiple replicates for the potential parents and sampling species from different geographical locations.

Author contributions

The authors confirm contribution to the paper as follows: Study conception and design: Henry RJ, Furtado A, Dillon NL; data collection: Wijesundara UK, Furtado A, Dillon NL, Masouleh AK; analysis and interpretation of results: Wijesundara UK, Furtado A, Dillon NL, Masouleh AK, Henry RJ; draft manuscript preparation: Wijesundara UK. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data supporting the findings of this study are available within the paper and its supplementary information. Raw Illumina sequence read data were submitted to NCBI's Sequence Read Archive (SRA) database under Bio project ID PRJNA940204 and under Bio sample ID's SAMN33621737-SAMN33621749 and SAMN40922882-SAMN40922886.

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Conflict of interest

The authors declare that they have no conflict of interest.

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