

RESEARCH

Open Access



Mitochondrial genome of the North American *Morus rubra* L. (red mulberry) reveals plastome gene transfer and essence of RNA editing

Bibek Adhikari¹ , Emily Ringgenberg¹, Gavin Smith¹, Ryan Johnson¹, Sanam Parajuli¹ , Jeffrey D. Carstens² and Madhav P. Nepal^{1*}

*Correspondence:

Madhav P. Nepal

Madhav.Nepal@sdstate.edu

¹Department of Biology and Microbiology, South Dakota State University (SDSU), Brookings, SD 57007, USA

²North Central Regional Plant Introduction Station (NCRPIS), United States Department of Agriculture – Agricultural Research Service (USDA-ARS), Ames, IA 50014, USA

Abstract

Morus rubra L. (Family: Moraceae), native to eastern North America, is distributed in the heart of pristine riparian forests and holds important ethnobotanical and ecological values. The species is severely threatened by introgressive hybridization with the introduced congener *Morus alba*, which is native to Asia. Insights into the mitochondrial (mito) genome of *M. rubra* along with comparative analyses against *M. alba* could potentially bridge the current knowledge gaps in understanding the genome architecture and hybridization patterns. The objectives of this study were to (1) sequence and assemble the draft mitogenome of *M. rubra*, and (2) perform a comparative mitogenomic phylogenetic analysis with the other 36 angiosperm taxa. *M. rubra* leaf sample from Waubesa State Park (Fremont County, Iowa) was sequenced for mitogenome analysis within the scope of the species' whole genome project. The mitogenome of *M. rubra* was 359,221 base pairs (bp) long with 45.8% GC content, comprising 57 genes: 32 protein-coding, 21 transfer, and four ribosomal RNAs. The chloroplast-to-mitogenome DNA transfer analysis revealed genes being synchronized with 17 homologous fragments from the chloroplast, accounting for 3.63% of the mitogenome. A total of 372 C to U RNA editing sites were detected in the mitochondrial protein-coding genes (mtPCGs) – responsible for the preprocessing of *rpl16* and *rps4* by adding a start codon, while postprocessing *atp9*, *ccmFN*, and *sdh4* by introducing a stop codon. The phylogenetic analysis of 37 species based on 23 shared mtPCGs revealed a tree topology identical to that proposed by the Angiosperm Phylogeny Group (APG) IV. This study is the first to report on the mitogenome of *M. rubra*, elucidating the mitogenome-based phylogeny and providing insights into the population genetics and evolution of mulberries. The publicly available *M. rubra* mitogenome enriches genomic resources for Moraceae and highlights the roles of SSRs, RNA editing, and inter-organellar DNA transfer in shaping mitogenome architecture.

Key message

Detailed analysis of the *Morus rubra* mitogenome provided insights into the genome with significant fragments inserted from the chloroplast and requirements for a few genes undergoing RNA pre- and post-processing.



Keywords Mitochondrial genome, *Morus rubra*, Red mulberry, *Morus* phylogeny, Phylogenomics of mulberries, Moraceae, Endangered species, RNA editing

1 Introduction

Morus rubra L. (Family: Moraceae), the red mulberry, is one of the 13 recognized *Morus* species and an endemic flora that maintains the biodiversity of the pristine riparian forests of North America [1–5]. The species' habitat ranges from the eastern margin of the Great Plains, extending north to southern Ontario, Canada, and distributed as far south as southern Florida [3, 4, 6]. The palatable fruit of this North American native plant is not only valued as a source of food, but Native Americans also use the plant's sap for the treatment of ringworm; tea made from leaves to treat and cure difficulty urinating, dysentery, and weakness [7–10]. Conservation assessments now flag a mounting concern: Michigan lists red mulberry as threatened [11]; Massachusetts lists red mulberry as endangered under the Massachusetts Endangered Species Act [12], Ontario lists it as endangered under provincial law [13], and NatureServe's 2025 review notes ongoing threats despite a global G5 rank—chiefly genetic swamping and habitat pressures [14].

M. rubra's species integrity has been threatened by introgressive hybridization with the introduced Asian congener *Morus alba* (white mulberry) [15–19]. *M. alba* was introduced to North America in the early colonial period (ca. 1600 AD) to establish a silk industry [20]. Despite the then government's efforts through perks and bounties, the attempts to establish the silk industry failed [21–23]. Nevertheless, this did not prevent the spread of white mulberries, which escaped the cultivated range, encroached and naturalized in the native red mulberries' habitats [4, 24]. The invasive congener soon started interweaving with the native population through hybridization, introducing fertile progeny, advancing to asymmetrical introgression with chloroplast (cp) capture and a nuclear bias toward *M. alba* alleles in sympatry [16–19]. This has not only challenged the conservation of *M. rubra*, but has also generated taxonomic confusions, even among experts, land managers, and other stakeholders [4]. This emphasizes the need for molecular markers to distinguish pure species and hybrids. Recent cp genome analyses of *M. rubra* revealed multiple haplotypes and highly variable regions, providing valuable tools for species identification, hybrid diagnosis, and conservation planning [5]. Understanding the underlying genetics of these two hybridizing species is essential for establishing conservation strategies for red mulberry. While the cp genome data have advanced our capacity to identify species and diagnose hybrids, the mitochondrial (mito or mt) genome remains unexplored in *M. rubra* and offers an additional layer of information for studying phylogenetics, organelle inheritance, and species integrity.

Mitochondria, also referred to as the “powerhouse of the cell”, predominantly provide cells with energy through oxidative phosphorylation occurring during the tricarboxylic acid (TCA) cycle [25]. They are double-membraned semi-autonomous organelles found across eukaryotes, comprising their own genome [26–28]. The endosymbiotic theory explains that once free-living prokaryotes evolved to form the mitochondrion as an autonomous eukaryotic cell organelle [29, 30]. Unlike in animals, where the mitogenome is circular and ranges from 15 to 17 kilobases (kb), the mitogenome size in plants varies greatly between similar, as well as within species [31–33]. Plant mitogenomes range from 66 kb in the parasitic *Viscum scurruloideum* [34] to 18.99 Mb in *Cathaya*

argyrophylla as reported by Huang et al. [35], surpassing the previously reported maximum of 11.7 Mb in Siberian larch *Larix sibirica* [36]. Despite the size of the mitogenome, it does not harbor a substantial number of genes [37]. The expanded size of the plant mitogenome is primarily due to the accumulation of repetitive sequences from the different organellar genomes: nuclear and chloroplast [38–41]. Moreover, gene transfer across mitochondria and different organellar genomes has been well-documented [42, 43]. Within *Morus*, mitogenome resources are emerging. Guo et al. [44] reported the mitogenome of *M. alba* var.- *atropurpurea* and *multicaulis* with a circular genome of 361,546 and 395,412 bp, respectively, accounting for ca. 7.80% cp to mt gene transfer. Building on our recent cp genome of *M. rubra* [5], we are now first to report *M. rubra* mitogenome, assembled from pair-ended short reads obtained from Illumina sequencing technology. These findings offer invaluable insights into the genetic, structural, and phylogenetic aspects of *M. rubra*, along with providing a baseline for investigating conservation approaches for the species.

2 Materials and methods

2.1 Sample collection and sequencing

The sample (Ames 35887 - #172–13) obtained through the U.S. National Plant Germplasm System [45] used in this study was collected from Waubonsie State Park, Fremont County, Iowa [Coordinates: 40.674843 N, 95.689384 W] on July 12, 2022. The plant identification was carried out following Nepal and Purinton [4] and also confirmed by Dr. Gary Larson – former curator of South Dakota State University's C.A. Taylor Herbarium (SDC) and Dr. Madhav Nepal, a *Morus* expert. The specimen voucher has been deposited at SDC as well as a duplicate at Iowa State University's Ada Hayden Herbarium (ISC) with voucher number #172–13. DNA extraction and 150 bp Illumina paired-end sequencing was carried out as described in Adhikari et al. [5]. Supplementary File 1 shows the leaf, fruits, and voucher of the specimen used.

2.2 Mitogenome assembly and annotation

The raw reads were subjected to quality control using FastQC [46] version 0.11.9, adapter trimming using Trimmomatic [47] version 0.39, and final quality assurance visualization using MultiQC [48] version 1.13. The parameters of Trimmomatic applied for read trimming include ILLUMINACLIP: TruSeq2-PE.fa:2:30:10:2:True for adapter removal, SLIDINGWINDOW:4:15 for quality filtering, LEADING:3 and TRAILING:3 for trimming low-quality bases, and MINLEN:36 to discard short reads. The curated reads were subjected to *de novo* mitogenome assembly using GetOrganelle [49] version 1.7.7.1, where '-F embplant_mt' was selected as seed with the program's default k-mer ('-k 21,55,85,115') values while running for 20 rounds ('-R 20'). The analysis script is also available on the author's GitHub repository [50]. For annotation of protein-coding genes (PCGs) in the mitogenome using Geseq [51] version 2.03, we selected *Arabidopsis thaliana* (NC037304), *Cannabis sativa* (NC029855), and *Morus notabilis* (NC041177) as reference genomes. Annotation of tRNA and rRNA-coding genes within the mitogenome was accomplished using tRNAscan-SE [52] version 2.0.7. BLAT [53] search with protein, rRNA, tRNA, and DNA search identity of 50 was also employed. Annotation errors in the genome were manually corrected using Artemis [54] version 18.2.0, and

OrganellarGenomeDRAW (OGDRAW) [55] version 1.3.1 was used for visualization of the genome.

2.3 Repeated sequences and codon usage analysis

The PCGs were subjected to Relative Synonymous Codon Usage (RSCU) analysis through the PhyloSuite [56] version 1.2.3 program, while graphical visualization was carried out in Microsoft Excel 2024.

The mono-, tri-, tetra-, penta-, hexa-, and hepta- nucleotide repeats were detected using the MicroSatellite Identification Tool (MISA) [57] version 2.1. The parameters were set to ten for mono-, five for di-, four for tri-, and three for tetra-, penta-, hexa-, and hepta-nucleotide repeats. The maximum length of a sequence between two simple sequence repeats (SSRs) to register as a compound SSR was set to 50 nucleotides. For the detection of forward (f), reverse (r), complement (c), and palindromic (p) repeats, a web-based program, REPuter [58] version 1 with default parameters to detect the repeats, ranging from 20 to 100 bases, was employed. The results were plotted in Microsoft Excel.

Tandem Repeats Finder (TRF) [59] version 4.09, with the criterion set to Basic: Use default parameters, was used to identify the Tandem Repeats.

2.4 Prediction of RNA editing sites

The C- to -U RNA editing sites in the mitochondrial PCGs (mtPCGs) were detected using Deepred-Mt [60] with default parameters. Only results exceeding the probability value of 0.9 were considered. Microsoft Excel's graphing function was used to visualize the results.

2.5 Chloroplast to mitochondrial DNA transformation

The chloroplast genome of the same sample we assembled earlier (NCBI GenBank Accession: PQ309081) [5] was employed for the chloroplast-to-mitochondrial DNA transfer analysis. NCBI-blast [61] version 2.14.1+, accessed through the High-Performance Computing cluster at South Dakota State University, was used to compare *M. rubra*'s organellar genomes. The cp genome was used as a query, whereas the mitogenome was used as a database, and the remaining parameters were set as default. The Advanced Circos program in TBtools-II (Toolbox for Biologists) [62] version 2.330 software was used to visualize regions of DNA transformation.

2.6 Synteny analysis

Reciprocal BLASTp of *M. rubra*'s mitochondrial proteome was carried out against other *Morus* mitogenomes, and the results were subjected to collinearity analysis using the Multiple Collinearity Scan toolkit (MCScanX) [63] version 1.0.0. The block size was defined as 5, with other parameters set as: match score 50, gap penalty -1, overlap window 5, e-value 1e-5, and maximum gaps 25. The collinear genes were visualized using the Multiple Synteny Plot tool built in TBTools-II.

2.7 Phylogenetic Inference

The mitogenomes of 35 species were retrieved from the NCBI GenBank [64], while the *M. rubra* and scaffold-level *M. alba* mitogenomes were assembled as part of this study.

The mitogenomes of the NCBI downloaded species were reannotated using GeSeq [51], and the mtPCGs were extracted from PhyloSuite [56]. The common 23 mitogenome genes, including *atp1*, *atp4*, *atp6*, *atp8*, *atp9*, *ccmB*, *ccmC*, *ccmFC*, *ccmFN*, *cob*, *cox1*, *cox2*, *cox3*, *matR*, *mttB*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, and *nad9*, were concatenated before multiple sequence alignment (MSA) carried through Multiple Alignment using Fast Fourier Transform (MAFFT) [65] version 7.525. The MSA output was subjected to Maximum Likelihood (ML) phylogenetic analysis with 1000 bootstrap (BS) replicates in IQTREE2 [66] version 2.3.1, selecting GTR + F + R4 as the best evolutionary model as determined by ModelFinder [67]. The ML tree file was converted to a Newick (.nwk) file in Interactive Tree of Life (iTOL) [68] version 7.2.1, while visualization was done in Molecular Evolutionary Genetic Analysis (MEGA) [69] version 12.

3 Results

3.1 Characteristics of the mitochondrial genome of *M. rubra*

As shown in Fig. 1, the draft mitogenome of *M. rubra* displayed a circular structure. The genome size was 359,221 bp with a GC content of 45.8%. The overall counts and percentage (in parentheses) of each nucleotide: A, T, G, and C in the mitogenome correspond to 96,869 (27.0), 97,823 (27.2), 82,377 (22.9), and 82,152 (22.9), respectively. We identified



Fig. 1 *Morus rubra* mitochondrial genome map. The dark gray internal concentric circle represents the GC content, while light gray represents AT content. The gray arrows show the downstream direction of the genes. The genes with '*' contain introns, while the genes in different colors denote the functional association of genes as defined by the accompanying legend

a total of 57 genes, including 32 PCGs, 17 unique tRNA genes – present in multiple copies (a total of 21 tRNAs), and four rRNA genes. The core angiosperm mitogenome genes consist of five ATP synthase (*atp1*, *atp4*, *atp6*, *atp8*, and *atp9*), nine NADH dehydrogenase (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, and *nad9*), four cytochrome C biogenesis (*ccmB*, *ccmC*, *ccmFC*, and *ccmFN*), three cytochrome C oxidase (*cox1*, *cox2*, and *cox3*), one protein transport subunit (*mttB*), one maturase (*matR*), and one ubiquinol-cytochrome C reductase (*cob*). The *nad1* and *nad5* genes were found to be trans-splicing genes. Additionally, the non-core genes include one ribosomal large subunit gene (*rpl16*) and six ribosomal small subunit genes (*rps3*, *rps4*, *rps7*, *rps12*, *rps13*, and *rps19*). There is also one succinate dehydrogenase gene (*sdh4*). Table 1 provides complete gene inventory, including copy numbers and intron information in the *M. rubra*'s mitogenome. The coding sequences (CDS) of PCGs include 28,363 bp, accounting for ca. 7.9% of the mitogenome, while the GC content is ca. 37.63%. Similarly, 3,787 bp (52.1% GC) encodes rRNAs, while 1,508 bp (50.6% GC) encodes tRNAs, accounting for ca. 1.05% and 0.42% of the entire genome, respectively. Among 32 PCGs, we found that CDS ranged from 198 bp (in *sdh4*) to 2,007 bp (in *nad5*), while GC content varied from 33% (in *nad4L*) to 53.1% (in *matR*). A total of 16 PCGs were detected on the positive (+) strand, 14 on the negative (-) strand, and two on the mixed (+/-) strand. Similarly, the nucleotide size for tRNAs ranged from 36 bp (in *trnR-ACG*) to 87 bp (in *trnS-GCU*), while GC ranged from 41.9% (in one of *trnM-CAU*) to 63.5% (in *trnD-GUC*). Eight of the tRNAs were distributed in the + strand, whereas 13 were found on the – strand of the mitogenome. Additionally, the size of rRNA and GC content ranged from 103 bp and 50.5% for *rrn4.5* to 1,936 bp and 53.4% for *rrn18*, respectively. The four rRNAs were proportionally distributed in the genome, two each in + and – strands. For detailed insights into the gene composition, see Supplementary File 2.

3.2 Repeat sequences in mitogenome

We identified diverse repeat motifs within the mitogenome, including mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide repeats as 41, 13, 12, 27, 1, and 1, respectively (see Fig. 2A). The most frequent mono-, di-, tri-, and tetra-nucleotide repeats (number in parentheses represents total # repeats) were A/T (37), AG/CT (11), AAG/CTT (6), and

Table 1 Annotated genes in the *Morus rubra* mitochondrial genome. The symbols ‘*’ and ‘x’ followed by a number, indicate intron-containing genes and number of gene copies, respectively.

Gene group	Gene names
ATP synthase	<i>atp1</i> , <i>atp4</i> , <i>atp6</i> , <i>atp8</i> , <i>atp9</i>
NADH dehydrogenase	<i>nad1</i> , <i>nad2</i> *, <i>nad3</i> , <i>nad4</i> *, <i>nad4L</i> , <i>nad5</i> *, <i>nad6</i> , <i>nad7</i> *, <i>nad9</i>
Cytochrome b	<i>cob</i>
Cytochrome c oxidase	<i>cox1</i> , <i>cox2</i> *, <i>cox3</i>
Cytochrome c biogenesis	<i>ccmB</i> , <i>ccmC</i> , <i>ccmFN</i> , <i>ccmFc</i> *
Maturases	<i>matR</i>
Protein transport subunit	<i>mttB</i>
Ribosomal protein large subunit	<i>rpl16</i>
Ribosomal protein small subunit	<i>rps3</i> , <i>rps4</i> , <i>rps7</i> , <i>rps12</i> , <i>rps13</i> , <i>rps19</i>
Succinate dehydrogenase	<i>sdh4</i>
Ribosomal RNA	<i>rrn4.5</i> , <i>rrn5</i> , <i>rrn18</i> , <i>rrn26</i>
Transfer RNA	<i>trnA</i> -UGC, <i>trnC</i> -GCA, <i>trnD</i> -GUC, <i>trnE</i> -UUC, <i>trnF</i> -AAA*, <i>trnF</i> -GAA, <i>trnG</i> -GCC, <i>trnI</i> -GAU, <i>trnK</i> -UUU, <i>trnM</i> -CAU (x4), <i>trnN</i> -GUU, <i>trnP</i> -UGG (x2), <i>trnQ</i> -UUG, <i>trnR</i> -ACG, <i>trnS</i> -GCU, <i>trnW</i> -CCA, <i>trnY</i> -GUA

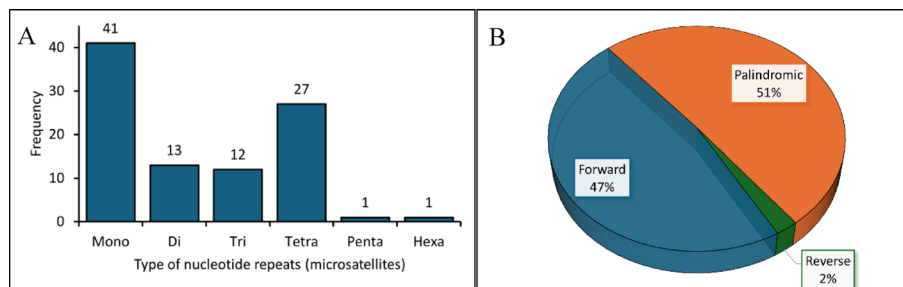


Fig. 2 Distribution of repeat sequences in the mitochondrial genome of *Morus rubra*. Panel (A) presents a bar graph showing the frequency of simple sequence repeats (SSRs), while panel (B) displays a pie chart illustrating the proportions of different repeat types identified in the mitogenome.

AAAC/GTTT (5), while TTAGG and GAAAGA were the penta- and hexa-nucleotides each with a single occurrence (See Supplementary File 3).

In addition, we found 178 repeats longer than 20 nucleotides, including 84 forward, 4 reverse, and 90 palindromic repeats. The pie chart in Fig. 2B illustrates the percentage distribution of the repeat types. No complement repeats were detected in the genome. A total of 17 tandem repeats were identified in the mitogenome, with the percentage match of repeats ranging from 79 to 100. The consensus size of the repeats ranged from 13 to 128 bp (See Supplementary File 4).

3.3 Codon usage in mitogenome

Codon usage analysis of 32 PCGs resulted in 9,084 codons, including 243 start and 52 stop codons (some subjected to RNA editing). The most frequently used codon was UUU (Phenylalanine, 330 occurrences), while the least frequently used stop codon was UGA (14 occurrences). Cysteine was the least frequent amino acid (141 codons, 1.56%), and Leucine was the most abundant (999 codons, 11.07%). The codon usage for each amino acid is presented in Supplementary File 5. With an RSCU value of 0.51, GAG encoding Glutamine was the least preferred codon, while GCU encoding Alanine was the preferred codon with an RSCU value of 1.65. Both AUG and UGG, coding for Methionine and Tryptophan, respectively, had an RSCU value of 1.0. Figure 3 shows the summary RSCU values for the used codon in the mitogenome of *M. rubra*. Additionally, the overall GC content of the PCGs was 37.63%.

3.4 RNA editing sites in the mitogenome

We predicted a total of 372 potential C-to-U (Cysteine-Uracil) RNA editing sites across 27 of 32 unique PCGs (cutoff ≥ 0.9). The highest number was detected in *nad7* (36 sites), followed by *mttB* (34), and *ccmB* (33). Also, *cox1* and *sdh4*, each had a single editing site. The *rps4* and *rpl16* genes lacking the start codon were subjected to modification through RNA editing. Similarly, the stop codons were added to the *atp9*, *ccmFN*, and *sdh4* genes. Figure 4 shows the distribution of the C-to-U RNA editing sites in the genes of *M. rubra* mitogenome. See Supplementary File 6. for details.

3.5 Chloroplast to mitochondrial gene transfer

Through sequence similarity analysis, we identified 17 homologous fragments between the mitogenome and cp genome, totaling 13,043 bp (Table 2). These fragments accounted for 3.63% of the entire mitogenome. When including inverted repeats from

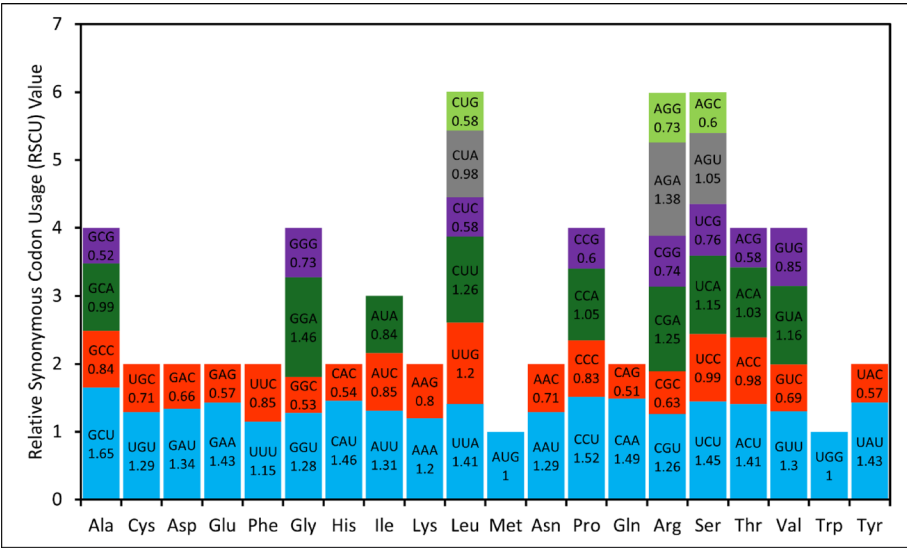


Fig. 3 *Morus rubra* mitogenome's relative synonymous codon usage (RSCU). The X-axis represents the amino acid with the associated codon and RSCU values on the Y-axis.

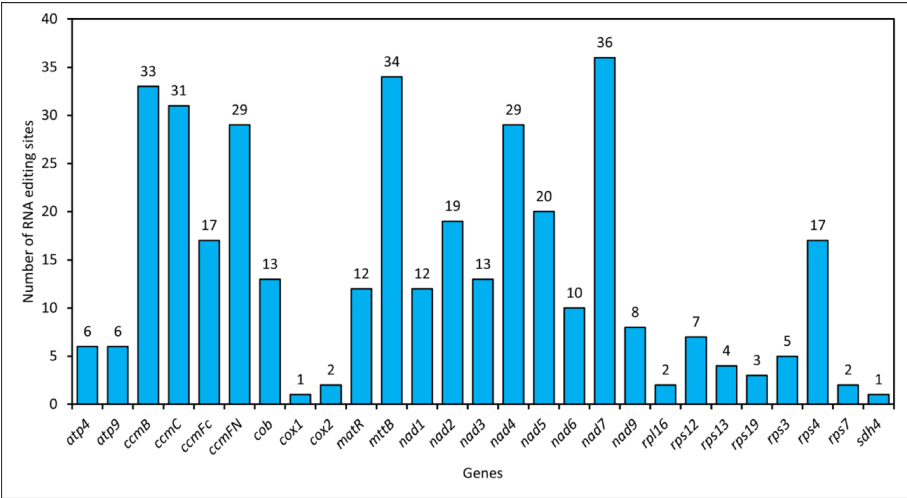


Fig. 4 Predicted C-to-U RNA editing sites in the genes of the *M. rubra* mitogenome (cutoff ≥ 0.9). Labels on the X-axis are the gene names, while the number of editable RNA sites is on the Y-axis.

the cp genome, the transfer accounted for 23,780 bp (6.62%). Most of these fragments migrated from chloroplast DNA (cpDNA) to mitochondrial DNA (mtDNA), except for a few tRNA genes that exhibited high sequence similarity, making it challenging to determine the direction of migration. The longest-spanning gene fragment was 5046 bp, while the smallest fragment was 43 bp long. The complete genes transferred between cp and mt genomes included ribosomal RNAs: *rrn4.5* and *rrn5*, while most other genes are transfer RNAs, including *trnA-UGC*, *trnI-GAU*, *trnM-CAU*, *trnW-CCA*, *trnN-GUU*, *trnP-UGG*, and *trnD-GUC*, whereas the transferred fragmented genes include *rbcl*, *psbA*, *psbC*, *rrn16*, *rpl2*, *rpl23*, *pafI*, *ndhB*, and *ycf2* (see Table 2). This finding suggests a notable sequence migration between the cpDNA and mtDNA of *M. rubra*, which was accompanied by gene transfer, a topic discussed in detail later. Figure 5 schematically

Table 2 The homologous sequences interchanged in the chloroplast and mitochondrial genomes of *M. rubra*.

S.N.	Percent Identity	Length (bp)	Mismatch	Gapopen	Cp start	Cp end	Mt start	Mt end	E-value	Bitscore	Associated gene(s)
1	99.881	5052	0	1	106,831	111,882	238,972	233,927	0	9291	<i>trnA-UGC/rnn23-frag/rnn4.5/rnn5/trnR-ACG-frag</i>
2	100	1717	0	0	140,859	142,575	43,200	41,484	0	3171	<i>trnI-GAU</i>
3	100	1691	0	0	88,646	90,336	58,478	56,788	0	3123	<i>rpl2-frag/rpl23-frag/trnM-CAU</i>
4	99.201	1127	0	1	151,177	152,303	104,968	103,851	0	2023	<i>ycf2-frag</i>
5	98.645	812	3	1	58,844	59,655	66,177	66,980	0	1432	<i>rbcL-frag</i>
6	91.264	538	16	6	810	1347	8495	9001	0	704	<i>psbA-frag</i>
7	79.528	508	65	23	70,077	70,562	123,337	122,847	3.83E-88	326	<i>trnW-CCA/trnP-UGG</i>
8	74.128	889	175	42	104,570	105,433	308,769	307,911	2.30E-85	316	<i>rnl16-frag</i>
9	93.59	156	5	2	59,617	59,771	54,013	53,862	1.11E-58	228	<i>rbcL-frag</i>
10	93.243	148	9	1	36,875	37,021	181,333	181,480	2.40E-55	217	<i>psbC-frag</i>
11	99.145	117	0	1	135,055	135,171	278,600	278,485	4.02E-53	209	<i>trnN-GUU</i>
12	97.561	82	1	1	32,478	32,559	325,102	325,182	5.35E-32	139	<i>trnD-GUC</i>
13	94.937	79	4	0	55,359	55,437	27,011	26,933	1.50E-27	124	<i>trnM-CAU</i>
14	90.805	87	8	0	93,360	93,446	122,383	122,297	2.51E-25	117	<i>ycf2-frag</i>
15	90.323	62	4	2	146,898	146,959	78,200	78,141	3.29E-14	80.5	<i>ndh8-frag</i>
16	95.652	46	0	1	46,052	46,097	70,630	70,673	5.50E-12	73.1	<i>psf1-frag</i>
17	95.349	43	2	0	89,640	89,682	67,014	66,972	7.12E-11	69.4	<i>rpl2-frag</i>

"Length (bp)" indicates the segment corresponding to the cp genome, while -frag is abbreviated for fragments transferred from the cp genome to the mitogenome.

illustrates the cp–mt gene transfers (i.e., homologs shared between the cp and mt genomes).

3.6 Synteny analysis

Figure 6 illustrates the varying arrangements of colinear blocks across the *Morus* mitogenomes. The major PCG pairs forming the collinear blocks include, *atp4-nad4L*, *ccmFN-mttB*, and *cox3-sdh4*, *nad3-rps12*, and *nad6-rps4*, respectively. Despite the PCGs being highly conserved across the sister species, our finding highlights the extensive rearrangement of the gene orders across mitogenomes.

3.7 Phylogenetic inference

The 23 conserved mtPCGs, including *atp1*, *atp4*, *atp6*, *atp8*, *atp9*, *ccmB*, *ccmC*, *ccmFC*, *ccmFN*, *cob*, *cox1*, *cox2*, *cox3*, *matR*, *mttB*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, and *nad9*, were used for phylogenetic analysis with *Amborella trichopoda* (Amborellales) as the outgroup. We found the phylogenetic tree topology similar to that of the Angiosperm Phylogeny Group (APG) IV [70]. We observed that Magnoliids and Monocots formed a single clade with a BS support of 87, which includes the orders Magnoliales (*Magnolia biondii* and *Liriodendron tulipifera*), Alismatales (*Spirodela polyrhiza*), Asparagales (*Crocus sativa* and *Asparagus officinalis*), Arecales (*Phoenix dactylifera* and *Cocos nucifera*), and Poales (*Triticum aestivum*, *Oryza sativa* Indica, *Zea mays*, and *Sorghum bicolor*). A 100 BS support was observed for the orders of monocots, while within monocots, the commelinids (Arecales and Poales) showed a common ancestry with a 98 BS support. Similarly, the dicots were found to be clustered together with a 100 BS support, where the basal eudicot order Ranunculales (*Pulsatilla dahurica* and *Aconitum kusnezoffii*) was found to be separating from a common ancestor of eudicots. Interestingly, the Vitales (*Vitis vinifera*) belonging to Rosid I/Fabidae was observed to be separated from the Rosid II/Malvidae by the insertion of Santalales (*Malaria oleifera* and *Santalum album*). We also observed that Rosid II formed a distinct clade with BS support of 62, comprising Fagales (*Betula pendula* and *Fagus sylvatica*) and Rosales, as indicated by green branches (see Fig. 7). Within Rosales, the order Moraceae (*M. rubra*, *M. notabilis*, *M. alba*, *M. alba* var.- *multicaulis*, and *atropurpurea*) formed a distinct clade with a BS support value of 100, shown in red. However, the North American *M. rubra* forming a cluster with non-sister *M. notabilis* seems to be interesting, albeit with a BS value of 57. The rest of the Rosales families included Rosaceae (*Prunus armeniaca* and *Malus domestica*), Rhamnaceae (*Ziziphus jujuba*), Ulmaceae (*Hemiptelea davidii*), and Cannabaceae (*Cannabis sativa*). Similarly, the Asterids' clade formed a distinct cluster with 100% BS support. Asterid II, Escalloniales (*Ilex rotunda*) and Asterales (*Helianthus annuus*) were found in isolation from Lamiales (*Lavandula angustifolia* and *Salvia miltiorrhiza*) and Solanales (*Nicotiana tabacum* and *Capsicum annuum*) of Asterid I. The clustering of Asterid I was supported with a BS value of 100. Figure 7 shows the phylogenetic tree based on the conserved mtPCGs across 37 species.

4 Discussion

The first mitochondrial genome to be sequenced was the human mitogenome in 1981 (~16 kb) [71], whereas the first plant mitogenome (~186 kb) was reported in 1992 from a liverwort, *Marchantia polymorpha* [72]. Since then, several species' mitogenomes

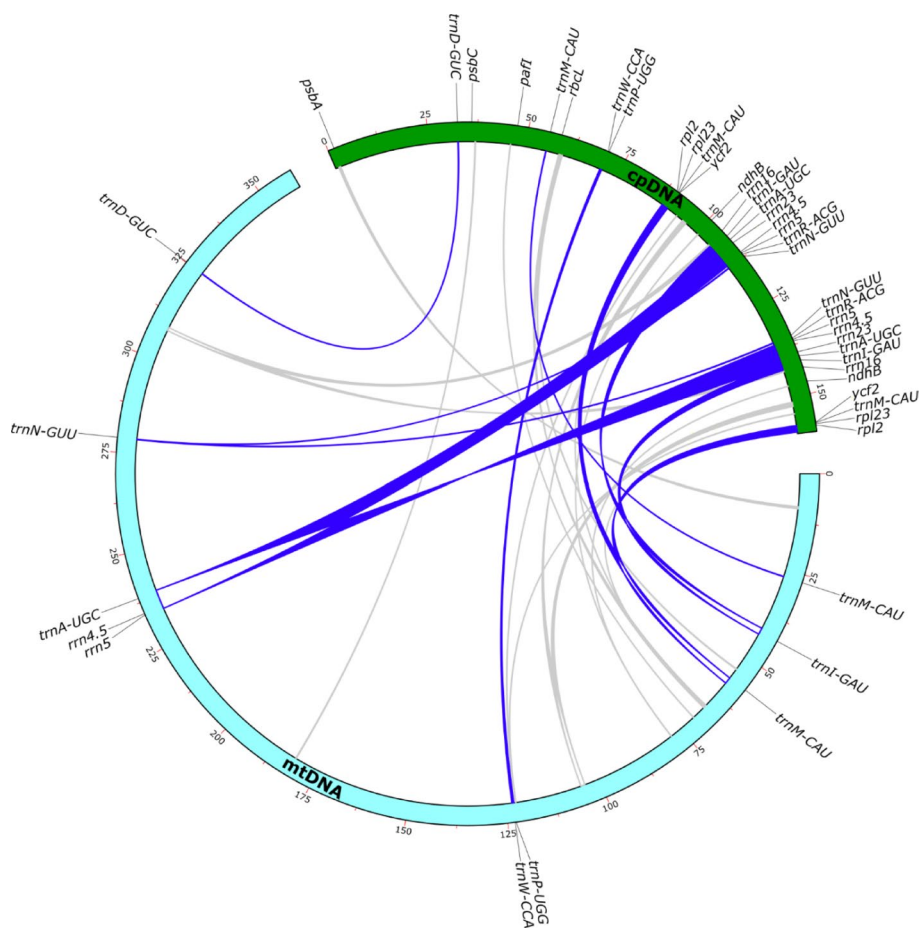


Fig. 5 Schematic representation of homologous sequences between the chloroplast and mitochondrial genomes in *M. rubra*. The cyan and green arcs represent the mitochondrial and chloroplast genomes. The internal curves indicate transferred fragments: dark blue denoting transferred complete genes, while gray represents gene fragments. The scale bars on the outer arcs depict 25 kb intervals

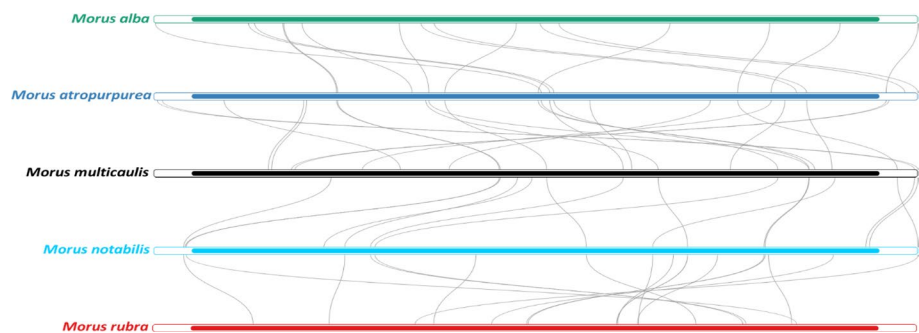


Fig. 6 Mitogenome synteny plot. Horizontal bars indicate the mitogenomes of different *Morus* species, while curves show homologous PCG sequences between adjacent species.

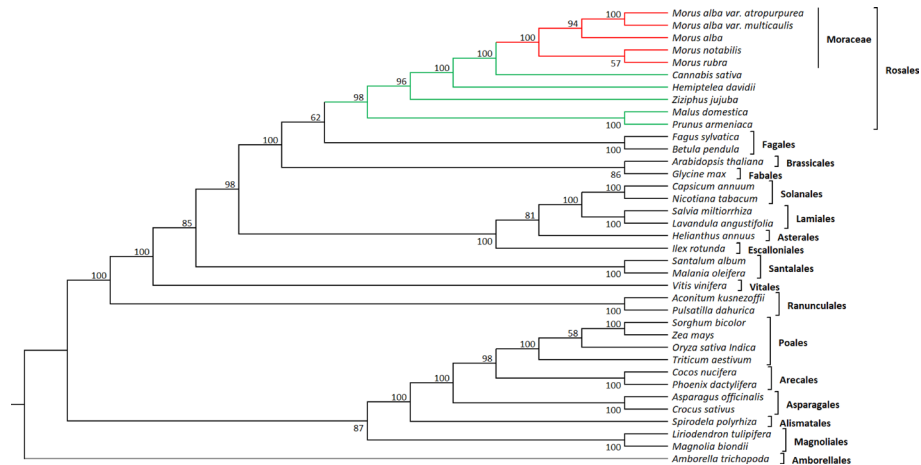


Fig. 7 Maximum-Likelihood phylogenetic tree based on 23 mitochondrial protein-coding genes (mtPCGs) illustrating the relationship among 37 different species, aligning with the Angiosperm Phylogeny Group (APG) IV classification. The phylogenetic analysis employed the GTR+I+G4 substitution model with 1000 bootstrap (BS) replicates. Numerical values along the branches indicate percentage BS support. Accessions marked with an asterisk (*) represent the genomes reported in this study.

Table 3 Comparative overview of four *Morus* mitogenomes.

Characteristics	<i>M. rubra</i> [*]	<i>M. alba</i> var. <i>atropurpurea</i> ^a	<i>M. alba</i> var. <i>multicaulis</i> ^a	<i>M. notabilis</i> ^a
GenBank Accession #	PX233331	MW924383	MW924382	NC041177
Genome size (bp)	359,221	395,412	361,546	362,069
Total number of genes	57	57	54	54
PCGs	32	32	31	30
tRNA	21	22	20	21
rRNA	4	3	3	3
GC content (%)	45.80	45.50	45.42	45.66

^{*}This study and ^aGuo et al. [44].

have been assembled. While animal mitogenomes are relatively smaller and highly conserved, plant genomes show great variations, including shape and size, varying even within the closely related species [31–33, 73, 74].

4.1 Characteristics of the *Morus* mitogenome

Within the genus *Morus*, *M. rubra* mitogenome is much smaller (359,221 bp) (Fig. 1) compared to that of *M. notabilis* (362,069 bp), *M. alba* var. *multicaulis* (361,546 bp), and *M. alba* var. *atropurpurea* (395,421 bp) [44]. The GC content in all four species was ca. 45%, while the total number of genes and PCGs detected in *M. rubra* and *M. alba* var. *atropurpurea* were 57 and 32, higher than the other two. *M. rubra* and *M. notabilis* both have 21 tRNAs, while the total number of genes encoding rRNAs is higher in *M. rubra* compared to other accessions [see Table 3 for details]. The mitogenome of *M. rubra* was ca. 2.27 times larger than its cp genome [5]. Across angiosperms mitogenome, 24 genes, including *atp1*, *atp4*, *atp6*, *atp8*, *atp9*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, *nad9*, *ccmB*, *ccmC*, *ccmFC*, *ccmFN*, *cox1*, *cox2*, *cox3*, *mttB*, *matR*, and *cob*, are considered core as they are unlikely to have undergone repetitive loss during the evolution across the lineage [75, 76]. The presence of these 24 genes in *M. rubra* indicates the conserved nature of the core mtPCGs. The CDS percentage in *M. rubra*'s mitogenome (ca. 7.9%) is larger than *Ficus carica* (ca. 5.42%) [77], *Lithocarpus litseifolius* (ca. 5.62%) [78], *Prunus*

tenella (ca. 6.77%) [79], *P. davidiana* (ca. 7.42%) [80], *Phaseolus vulgaris* (ca. 7.26%) [81], *M. alba* var- *multicaulis* (ca. 7.73%) and *atropurpurea* (ca. 7.14%) [44, 82]; while smaller than *Paeonia ludlowii* (ca. 8.60%) [83] and *Perilla frutescens* (ca. 11.37%) [84].

4.2 Repeat regions in *Morus* mitogenomes

Repeat regions, including tandem, long, and short sequence repeats, are common in the *Morus* mitogenome [44]. These sequences are known to play a significant role in mitochondrial genome rearrangement [82, 85]. Notably, the relatively higher number of SSRs and repeats were found in the *M. rubra* mitogenome (95 and 178, respectively) (Fig. 2) compared to its chloroplast genome (75 SSRs and 46 repeats), likely reflecting its larger genome size and extensive non-coding regions. Similar to that in the *M. rubra*'s cp genome, SSRs and repeats in the mitogenome were distributed across introns and inter-genic spacer regions [5]. Interestingly, the total SSRs detected in *M. alba* var. *multicaulis* are ca. four times more than in *M. rubra* [44], suggesting a wide interspecific variation in repeat content. It is important to emphasize that the choice of sequencing platform can substantially influence the detection of the SSRs. Third-generation technologies, such as Oxford Nanopore and PacBio, generate longer reads and provide more complete assemblies of mitochondrial genomes compared to second-generation short-read methods. This increased resolution allows for more accurate identification of repetitive regions, which in turn may account for the higher number of SSRs observed. Consequently, the four-fold difference reported here is likely attributable, at least in part, to methodological differences between sequencing approaches rather than to biological variation alone. In *M. rubra*'s mitogenome, SSRs were the most abundant repeats, aligning with reports in various other plants [42, 86]. Previously, SSRs from the pigeon pea (*Cajanus cajan*)'s mitogenome were used as molecular markers for genotype identification [87]. We believe that the diverse SSRs and tandem repeats detected in this study may serve as valuable genetic markers for DNA fingerprinting, studying population structure of *M. rubra* as well as phylogenetic relationships among species in the genus *Morus*.

4.3 Codon usage

RSCU is the relative frequency of synonymous codons used for encoding amino acids in a genome [88]. An RSCU value of less than one means rarity in the use of the codon, equal to one means unbiased use of the codon, while greater than one means preference of a specific codon over another [88]. Since methionine (AUG) and tryptophan (UGG) are both coded by their respective single designated codons, the RSCU value is equal to one as expected [88]. In this study, CGA (encoding Glutamine) showed the lowest RSCU value, and GCU (encoding Alanine) had the highest. These patterns align with Guo et al. [44]'s findings in *M. alba* var- *atropurpurea* and *multicaulis* mitogenomes, suggesting conserved codon usage bias across *Morus* species. Unlike 22,775 codons detected in the *M. rubra* cp genome [5], its mitogenome has codons reduced by two-fifths (9,084 codons in total) (Fig. 3), which aligns with the reduced number of PCGs (32 in the mitogenome and 83 in the chloroplast). The GC content of the PCGs in the mitogenome (37.63%) was observed to align with the reported value for the *M. rubra*'s chloroplast (~37%), indicating compositional consistency across organellar genomes. These findings provide insight into codon usage dynamics and genome architecture in *M. rubra*, and may be

implemented in future studies on translational efficiency, evolutionary constraints, and organelle-specific gene expression.

4.4 RNA editing and translational regulation

Proper folding of cp and mt proteins in higher plants is accomplished by the process of RNA editing, a crucial post-transcriptional modification tool [89]. The number of RNA editing sites in plants can vary, with the highest number recorded in the clubmoss *Selaginella moellendorffii* with 2,152 sites [90], while they can range between 300 to 500 in angiosperms (reviewed in [91]). In this study, we identified 372 C-to-U editing sites in the 27 PCGs with a confidentiality threshold above 0.9 (Fig. 4). The number of C to U editing sites in the mitogenome of *M. rubra* is comparable to that of *M. multicaulis* (377) [44], suggesting conserved post-translational regulation within the genus. A high binding free energy characterizes translational accuracy; thus, to regulate the molecular function, the G/C codon, with a higher binding affinity, is preferred through RNA editing [92, 93]. In *M. rubra*, RNA editing is required to create start codons in *rpl16* and *rps4* for translation initiation, and to generate stop codons in *atp9*, *ccmFN*, and *sdh4* for transcript termination. This finding is consistent with observations in other plant species such as *Meniocus linifolius*, where the start codon is introduced in *rpl16*, while in *Crucihimalaya lasiocarpa* and *Lepidium sativum*, the stop codon is introduced in the *ccmFN* genes through RNA editing [94]. Similarly, in *Arabidopsis thaliana*, two exonucleases act simultaneously to process the 3'-end of *atp9* mRNA, as it lacks a stop codon [95]. Handa et al. [96] reported the need of RNA editing for the expression of the *rps4* gene in *Rubus* sp. and *Oryza sativa*. Additionally, in *Solanum tuberosum*, the *sdh4* gene undergoes RNA editing along with co-transcription with the *cox3* gene [97]. Varré et al. [98] also reported the requirement of RNA editing to create a stop codon for transcriptional termination of *atp9* in *S. tuberosum*. Together, these findings highlight the conserved and dynamic role of RNA editing in regulating mitochondrial gene expression.

4.5 Chloroplast-to-mitochondria DNA transfer

The *M. rubra* mitogenome demonstrated gene remodeling with the incorporation of ~13 kb fragments from its cp genome. Such inter-organellar gene transfers have been widely reported across plant species, including *Stemona sessilifolia* [42], *Morus alba* var. *multicaulis* [44], *Apostasia shenzhenica* [86], and *Camellia sinensis* [99]. Our analysis revealed a significant proportion of cp to mt DNA transfer, accounting for a total of 3.63% of the sequenced genome (Fig. 5; Table 2). Previously, Guo et al. [44] reported ~7.80% DNA migration in *Morus* species, while Lai et al. [100] reported ca. 6–10% of the entire genome in closely related three accessions of *Broussonetia* species. Complete gene transfers from cp to mt genome are well documented in angiosperms, mainly comprising tRNA genes, including *trnA-UGC*, *trnI-GAU*, *trnM-CAU*, *trnW-CCA*, *trnN-GUU*, *trmP-UGG*, and *trnD-GUC* [89, 101]. Similarly, the transfer of rRNA genes like *rrn4.5* and *rrn5* has been previously reported in a seagrass, *Zostera caespitosa* [102]. Nevertheless, migration of fragmented genes along with the larger chunk of DNA from the cp to the mt genome is highly significant too. Tang et al. [103] reported the transfer of *rpl2* and *rrn16* fragments in *Paeonia lactiflora*, which aligns with our results. Recent comparative analyses in sweet potato (*Ipomoea batatas*) further support the prevalence of horizontal gene transfer (HGT) between organelles, revealing 33 mitochondrial segments with high

homology to chloroplast sequences [104]. These findings reinforce the evolutionary significance of cp-to-mt DNA migration, which has been occurring in angiosperms since at least 300 million years ago (MYA), during the Carboniferous period [105].

4.6 Synteny and conserved PCG clusters

Synteny analysis of the PCGs in *M. rubra* mitogenome revealed extensive rearrangements, including cpDNA fragment incorporated as foreign sequences and inversions (Fig. 6). These rearrangements reflect the dynamic nature and are consistent with patterns observed in other angiosperms. In *M. rubra*, *atp4-nad4L* and *nad3-rps12* are among the ancestral conserved gene clusters, as reported in *A. thaliana* [106], *Cryptocarya kwangtungensis* [107], and *Ajuga reptans* [108] earlier. The *cox3-sdh4* gene cluster, along with being ancestrally conserved collinear genes, also engages in co-transcription for transcript termination of *sdh4* as it lacks a stop codon [97]. The gene pairs *nad6-rps4* and *ccmFN-mttB* are newly reported in mulberries in this study. As previously reported by Huang et al. [107], an interlocking pattern was exhibited in the conserved gene clusters in addition to being encoded in the same direction. Moreover, the presence of highly conserved tRNAs compared to the PCGs indicates their crucial importance in the genome [44].

4.7 Phylogenetic relationships and taxonomic implications

The mitogenome structure in plants continues to evolve dynamically, exhibiting substantial variation in size, gene content, and structural rearrangements; however, it has a slower rate of nucleotide substitution [31–33, 109]. In contrast, the cp genome has a higher evolution rate and is supposed to have a lack of recombination, making it suitable for phylogenetic studies [110]. Due to this reason, the mitogenome is historically underutilized in phylogenetic studies, particularly at the species and genus levels [111]. However, recent large-scale phylogenomic analyses have demonstrated that mtPCGs can effectively resolve deep node relationships across angiosperms, offering robust support for major clades and providing complementary insights to plastid and nuclear datasets. These findings are consistent with our results (Fig. 7) and underscore the growing relevance of mitogenomic data in evolutionary biology, especially for taxa with complex or ambiguous histories [76].

In our study, phylogenetic reconstruction based on *M. rubra* mtPCGs yielded a tree topology (see Fig. 7) consistent with the APG IV classification [70], reinforcing the reliability of mitochondrial markers for interspecific analyses. The nesting pattern of the *Morus* species in the phylogenetic tree mostly aligns with the evolutionary perspectives previously reported based on chloroplast and nuclear gene regions [4, 5, 112]. The clustering of *M. alba*, *M. alba* var. *- atropurpurea*, and *multicaulis* was expected, as the latter two are varieties of the former. In contrast, the nesting of *M. notabilis* with *M. rubra* was an interesting observation. The unexpected nesting of *M. notabilis* with *M. rubra*, however, suggests a possible earlier divergence event, a hypothesis supported by chloroplast-based phylogenies and divergence time estimates [5]. The genus *Morus* includes 13 recognized species distributed worldwide [4], yet mitogenomic data remain available for only a subset. Expanding mitogenome sequencing to include all *Morus* species would be instrumental in refining phylogenetic relationships and resolving taxonomic ambiguities. Notably, the current BS support for the *M. rubra*–*M. notabilis* clade is

relatively weak (57), indicating that additional data could significantly alter tree topology and improve resolution. Phylogenetic analysis by Yang et al. [113] placed *M. notabilis* as sister to both American and Asian clades with strong bootstrap support (BS = 100). In contrast, our mitochondrial phylogeny shows a weak clustering of *M. rubra* and *M. notabilis* (BS = 57), which does not align with the well-supported chloroplast phylogenies that consistently separate *M. rubra* into the North American clade and *M. notabilis* into the Asian clade. This discrepancy highlights interesting evolutionary signals between organellar genomes. Hybridization and introgression, which are frequently reported in *Morus* species across both American and Asian lineages, may have contributed to conflicting phylogenetic patterns. Additionally, incomplete lineage sorting could explain the weak mitochondrial support for clustering, as ancestral polymorphisms may persist differently in mt versus cp genomes. Together, these factors suggest that mitochondrial phylogenies should be interpreted cautiously and in conjunction with chloroplast and nuclear data to fully resolve the evolutionary history of *Morus*. Furthermore, with the continuous advancement of phylogenetic tools—such as model-aware partitioning, site-heterogeneous substitution models, and improved alignment algorithms—we strongly recommend reannotation and quality control of publicly available organellar genomes. This step is essential to minimize the risk of false-positive phylogenies and to ensure reproducibility and accuracy in evolutionary studies.

In summary, while mitogenomes have traditionally been overlooked in plant systematics, emerging evidence and analytical innovations are rapidly elevating their status as valuable resources for phylogenetic reconstruction, especially when integrated with plastid and nuclear data.

5 Conclusion

We assembled and annotated the mitochondrial genome of *Morus rubra*, revealing a circular structure of 359,221 bp with ~45.8% GC content and 57 genes (32 PCGs, 21 tRNAs, and four rRNAs). The genome is ~2.3 times larger than its chloroplast counterpart and contains numerous repeats, which likely contribute to structural complexity. Codon usage showed conserved patterns across *Morus*, and 372 RNA editing sites were detected, essential for generating start and stop codons in several genes. Chloroplast-to-mitochondria DNA transfer accounted for ~3.6% of the mitogenome, and phylogenetic analysis based on 23 PCGs supported APG IV topology, though *M. rubra* clustered weakly with *M. notabilis* (BS = 57), suggesting possible historical introgression or incomplete lineage sorting.

Importantly, differences in SSR counts among *Morus* species may reflect sequencing platform effects rather than biological variation alone. Third-generation technologies (e.g., Oxford Nanopore, PacBio) produce longer reads and more complete assemblies than short-read Illumina sequencing, enabling better detection of repetitive regions. This methodological factor likely explains the four-fold SSR difference observed in the present study.

Overall, the *M. rubra* mitogenome enriches Moraceae genomic resources and highlights the roles of repeats, RNA editing, and inter-organellar DNA transfer in mitochondrial genome evolution.

6 Authors' note

The plant species with their mitogenomes' NCBI GenBank accession numbers (in parentheses) analyzed in this research article include: *Amborella trichopoda* (KF754803), *Magnolia biondii* (NC049134), *Spirodela polyrhiza* (NC017840), *Crocus sativus* (OL804177), *Asparagus officinalis* (NC053642), *Phoenix dactylifera* (NC016740), *Cocos nucifera* (NC031696), *Triticum aestivum* (NC036024), *Oryza sativa* var. *indica* (NC071219), *Zea mays* (NC008332), *Sorghum bicolor* (NC008360), *Pulsatilla dahurica* (NC071219), *Aconitum kusnezoffii* (NC053920), *Vitis vinifera* (NC012119), *Malania oleifera* (MT902145), *Santalum album* (OQ868374), *Ilex rotunda* (NC084321), *Heli-anthus annuus* (KF815390), *Lavandula angustifolia* (OR296704), *Salvia miltiorrhiza* (NC023209), *Nicotiana tabacum* (NC006581), *Capsicum annuum* (NC024624), *Glycine max* (JX463295), *Arabidopsis thaliana* (NC037304), *Betula pendula* (LT855379), *Fagus sylvatica* (MT446430), *Prunus armeniaca* (NC065228), *Malus domestica* (MN964891), *Ziziphus jujuba* (NC029809), *Hemiptelea davidii* (MN061667), *Cannabis sativa* (NC029855), *Morus rubra* (PX233331), *Morus notabilis* (NC041177), *Morus alba* (PX243397), *Morus alba* var. *atropurpurea* (MW924383), and *Morus alba* var. *multicaulis* (MW924382).

Abbreviations

DNA	Deoxyribonucleic acid(s)
RNA	Ribonucleic acid(s)
PCG	Protein coding gene(s)
tRNA	Transfer ribonucleic acid(s)
rRNA	Ribosomal ribonucleic acid(s)
mito or mt	Mitochondrial/mitochondrion
cp	Chloroplast(s)
bp	Base pair(s)
TCA	Tricarboxylic acid
SSR	Simple sequence repeat(s)
CDS	Coding sequence(s)
BS	Bootstrap
RSCU	Relative synonymous codon usage

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44372-026-00476-5>.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Acknowledgements

The authors would like to express sincere gratitude to Andrew Sherwood from the North Central Regional Plant Introduction Station (NCRPIS), United States Department of Agriculture – Agricultural Research Service (USDA-ARS), Ames, Iowa, for their support with field assistance and plant sampling. The authors acknowledge South Dakota State University (SDSU)'s Functional Genomics Core Facility for equipment support for DNA work and thank High Performance Computing (HPC) at SDSU for providing the computational resources for completion of the project. A sincere thanks to the Department of Biology and Microbiology, SDSU, for their continuous support.

Author contributions

B.A. performed the experiments, analyses, wrote the scripts and codes, and wrote and reviewed the original manuscript. E.R., G.S., and R.J. assisted in laboratory tasks. S.P. assisted in writing and reviewing the draft. J.D.C. assisted with field sampling and contributed to revising the manuscript. M.P.N. conceived and supervised the project, framed the study and analyses, and assisted in writing, reviewing, and finalizing the manuscript. All authors read and approved the final version of the manuscript.

Funding

The USDA-AFRI (Award #2022-67037-36254) and South Dakota Agriculture Experiment Station Hatch Project #SD00H800-23 to M. P. Nepal supported this research work.

Data availability

The mitochondrial genomes assembled in this study, with accession numbers **PX233331** (<https://www.ncbi.nlm.nih.gov/nuccore/PX233331.1>) for *Morus rubra* and **PX243397** (<https://www.ncbi.nlm.nih.gov/nuccore/PX243397.1>) for *Morus alba*, have been deposited into the NCBI GenBank repository.

Code availability

The codes and scripts used in this study are publicly available from the author's GitHub repository at https://github.com/abibek52/Morus_rubra_mitogenome_project [50].

Declarations

Ethics approval and consent to participate

Although *Morus rubra* is not listed as threatened or endangered at the collection site, we followed the International Union for Conservation of Nature (IUCN) Policy Statement guidelines for the collection of plant materials. Permission to collect plant leaf samples from Waubonsie State Park, Fremont County, Iowa, through the Iowa Department of Natural Resources (DNR), was obtained by co-author Jeffrey D. Carstens.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 28 October 2025 / Accepted: 14 January 2026

Published online: 09 February 2026

References

1. Burgess KS. (2004). The genetic and demographic consequences of hybridization in small plant populations. PhD Dissertation, The University of Guelph. Available at: <https://hdl.handle.net/10214/20154>
2. Nepal MP. (2008). Systematics and reproductive biology of the genus *Morus* L. (Moraceae). PhD Dissertation, Kansas State University. Available at: <https://www.proquest.com/dissertations-theses/systematics-reproductive-biology-genus-i-morus-l/docview/304563616/se-2?accountid=28594>
3. Nepal M, Wichern DJ. (2013). Taxonomic status of red mulberry (*Morus rubra*, Moraceae) at its Northwestern boundary. Proceedings of the South Dakota Academy of Science 92:19–29. Available at: <https://research.ebsco.com/c/q775jt/search/details/okccauo7pn?db=f6h>
4. Nepal MP, Purinton JM. (2021). Systematics of the genus *Morus* L. (Moraceae): taxonomy, phylogeny and potential responses to climate change. Mulberry: Genetic Improvement in Context of Climate Change. CRC Press Available at: <https://books.google.com/books?id=EJgsEAAQBAJ>
5. Adhikari B, Parajuli S, Nepal MP. Reporting complete chloroplast genome of endangered red mulberry, useful for understanding hybridization and phylogenetic relationships. Sci Rep. 2025;15:13403. <https://doi.org/10.1038/s41598-025-97618-8>.
6. Parks Canada Agency. (2011). Recovery strategy for the red mulberry (*Morus rubra*) in Ontario. Species at Risk Act recovery strategy series. Parks Canada Agency, Ontario Ministry of Natural Resources, Cornwall, Ontario. Available at: https://registrelep-sararegistry.gc.ca/virtual_sara/files/plans/rs_red_mulberry_0613_e.pdf
7. Carlson GG, Jones VH. Some notes on uses of plants by the Comanche Indians. Papers Mich Acad Sci Arts Lett. 1939;25:517–42. <https://ehrafworldcultures.yale.edu/document?id=no06-005>.
8. Hamel PB, Chiltoskey MU. (1975). Cherokee plants and their uses: a 400 year history. Herald Pub. Co., Sylva, NC. Available at: <https://ehrafworldcultures.yale.edu/document?id=nn08-003>
9. Moerman DE. (1998). Native American ethnobotany. Timber Press, Portland, Oregon. Available at: <https://search.library.wisc.edu/catalog/999840805302121>
10. Foster S, Duke JA. A field guide to medicinal plants: Eastern and central North America. The Peterson field guide series (USA). Boston: Houghton Mifflin; 1990.
11. Penskar MR. (2009). Special plant abstract for *Morus rubra* (red mulberry). Michigan Natural Features Inventory, Lansing, MI Available at: https://mnfi.anr.msu.edu/abstracts/botany/Morus_rubra.pdf
12. Mass.gov. (2025). Red mulberry: A species of greatest conservation need in the MA state wildlife action plan. Available at: <https://www.mass.gov/info-details/red-mulberry>
13. Ontario. (2014). Red mulberry (Species at risk). Available at: <https://www.ontario.ca/page/red-mulberry-species-risk>
14. NatureServe. (2025). *Morus rubra*: Red mulberry. Available at: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.137019/Morus_rubra
15. Salah SM. (2006). Genetic and morphological characterization of red mulberry (*Morus rubra* L.), white mulberry (*Morus alba* L.) and their hybrids. M.S. Thesis, Central Missouri State University. Available at: <https://www.proquest.com/dissertations-theses/genetic-morphological-characterization-red/docview/304919379/se-2?accountid=28594>
16. Burgess KS, Husband BC. Maternal and paternal contributions to the fitness of hybrids between red and white mulberry (*Morus*, Moraceae). Am J Bot. 2004;91:1802–8. <https://doi.org/10.3732/ajb.91.11.1802>.
17. Burgess KS, Morgan M, Deverno L, Husband BC. Asymmetrical introgression between two *Morus* species (*M. alba*, *M. rubra*) that differ in abundance. Mol Ecol. 2005;14:3471–83. <https://doi.org/10.1111/j.1365-294x.2005.02670.x>.

18. Burgess KS, Husband BC. Habitat differentiation and the ecological costs of hybridization: the effects of introduced mulberry (*Morus alba*) on a native congener (*M. rubra*). *J Ecol.* 2006;94:1061–9. <https://doi.org/10.1111/j.1365-2745.2006.01152.x>.
19. Burgess KS, Morgan M, Husband BC. Interspecific seed discounting and the fertility cost of hybridization in an endangered species. *New Phytol.* 2008;177:276–84. <https://doi.org/10.1111/j.1469-8137.2007.02244.x>.
20. Klose N. (1963). Sericulture in the United States. *Agricultural History* 37:225–234. Available at: <https://jstor.org/stable/3739985>
21. Brockett LP (1876). The silk industry in America: a history. George F. Nesbitt & Company. Available at: <https://archive.org/details/silkindustryina00brocgoog/page/n4/mode/2up>
22. Matsui S. (1927). The history of the silk industry in the United States. PhD Dissertation, The University of Wisconsin-Madison. Available at: https://books.google.com/books?hl=en&lr=&id=TYVPAAMAAJ&oi=fnd&pg=PA63&dq=The+history+of+the+silk+industry+in+the+United+States&ots=wYDw6hhLrP&sig=j7x7i9P7nMO9YfZkMuXey_r4_Kc#v=onepage&q=The%20history%20of%20the%20silk%20industry%20in%20the%20United%20States&f=false
23. Hatch CE. (1957). Mulberry trees and silkworms: Sericulture in early Virginia. *The Virginia Magazine of History and Biography*. Virginia Historical Society Available at: <http://www.jstor.org/stable/4246279>
24. Wunderlin RP. *Moraceae. Flora of North America North of Mexico*. New York: Oxford University Press; 1997. pp. 388–92.
25. Anderson AJ, Jackson TD, Stroud DA, Stojanovski D. Mitochondria-hubs for regulating cellular biochemistry: emerging concepts and networks. *Open Biol.* 2019;9:190126. <https://doi.org/10.1098/rsob.190126>.
26. Sjöstrand FS. (1956). The ultrastructure of cells as revealed by the electron microscope. In: Bourne GH, Danielli JF, editors *International Review of Cytology*. Academic Press, pp 455–533. [https://doi.org/10.1016/S0074-7696\(08\)62578-4](https://doi.org/10.1016/S0074-7696(08)62578-4)
27. Mahler HR. (1973). Genetic autonomy of mitochondrial DNA. In: Hamkalo BA, Papaconstantinou J, editors *Molecular Cytogenetics*. Springer New York, Boston, Massachusetts, pp 181–208. https://doi.org/10.1007/978-1-4615-7479-8_15
28. Saccone C, Gissi C, Lanave C, Larizza A, Pesole G, Reyes A. Evolution of the mitochondrial genetic system: an overview. *Gene.* 2000;261:153–9. [https://doi.org/10.1016/S0378-1119\(00\)00484-4](https://doi.org/10.1016/S0378-1119(00)00484-4).
29. Wallin IE. (1927). *Symbiontism and the origin of species*. Williams & Wilkins Company, Baltimore, Maryland. <https://doi.org/10.5962/bhl.title.11429>
30. Zimorski V, Ku C, Martin WF, Gould SB. Endosymbiotic theory for organelle origins. *Curr Opin Microbiol.* 2014;22:38–48. <https://doi.org/10.1016/j.mib.2014.09.008>.
31. Kubo T, Newton KJ. Angiosperm mitochondrial genomes and mutations. *Mitochondrion.* 2008;8:5–14. <https://doi.org/10.1016/j.mito.2007.10.006>.
32. Allen JO, Fauron CM, Minx P, Roark L, Oddiraju S, Lin GN, Meyer L, Sun H, Kim K, Wang C. Comparisons among two fertile and three male-sterile mitochondrial genomes of maize. *Genetics.* 2007;177:1173–92. <https://doi.org/10.1534/genetics.107.073312>.
33. Gualberto JM, Mileshina D, Wallet C, Niazi AK, Weber-Lotfi F, Dietrich A. The plant mitochondrial genome: dynamics and maintenance. *Biochimie.* 2014;100:107–20. <https://doi.org/10.1016/j.biochi.2013.09.016>.
34. Skippington E, Barkman TJ, Rice DW, Palmer JD. Miniaturized mitogenome of the parasitic plant *viscum scurruloideum* is extremely divergent and dynamic and has lost all Nad genes. *Proc Natl Acad Sci.* 2015;112(E3515–E3524). <https://doi.org/10.1073/pnas.1504491112>.
35. Huang K, Xu W, Hu H, Jiang X, Sun L, Zhao W, Long B, Fan S, Zhou Z, Mo P, Jiang X, Tian J, Deng A, Xie P, Wang Y. Super-large record-breaking mitochondrial genome of *Cathaya argyrophylla* in Pinaceae. *Front Plant Sci.* 2025;16:1556332. <https://doi.org/10.3389/fpls.2025.1556332>.
36. Putintseva YA, Bondar EI, Simonov EP, Sharov VV, Oreshkova NV, Kuzmin DA, Konstantinov YM, Shmakov VN, Belkov VI, Sadovsky MG. Siberian larch (*Larix sibirica* Ledeb.) mitochondrial genome assembled using both short and long nucleotide sequence reads is currently the largest known mitogenome. *BMC Genomics.* 2020;21:1–12. <https://doi.org/10.1186/s12864-020-07061-4>.
37. Zardoya R. Recent advances in understanding mitochondrial genome diversity. *F1000Res.* 2020;9. <https://doi.org/10.12688/f1000research.21490.1>.
38. Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Mol Ecol.* 2005;14:689–701. <https://doi.org/10.1111/j.1365-294X.2004.02410.x>.
39. Greiner S, Bock R. Tuning a ménage à trois: co-evolution and co-adaptation of nuclear and organellar genomes in plants. *BioEssays.* 2013;35:354–65. <https://doi.org/10.1002/bies.201200137>.
40. Tan J, Forner J, Karcher D, Bock R. DNA base editing in nuclear and organellar genomes. *Trends Genet.* 2022;38:1147–69. <https://doi.org/10.1016/j.tig.2022.06.015>.
41. Chu L, Du Q, Zuo S, Liu G, Wang H, Liu G, Zhao L, Xu G. Assembly and comparative analysis of the complete mitochondrial genome of *Vaccinium carlesii* Dunn. *Genomics.* 2024;116:110897. <https://doi.org/10.1016/j.ygeno.2024.110897>.
42. Xie Y, Liu W, Guo L, Zhang X. Mitochondrial genome complexity in *Stemona sessilifolia*: nanopore sequencing reveals chloroplast gene transfer and DNA rearrangements. *Front Genet.* 2024;15:1395805. <https://doi.org/10.3389/fgene.2024.1395805>.
43. Adams KL, Palmer JD. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol Phylogenet Evol.* 2003;29:380–95. [https://doi.org/10.1016/S1055-7903\(03\)00194-5](https://doi.org/10.1016/S1055-7903(03)00194-5).
44. Guo L, Shi Y, Wu M, Michael A, Guo P, Zheng D, Qiang L, Weiguo Z. The complete mitochondrial genome sequence variation and phylogenetic analysis of mulberry. *Res Square.* 2021. <https://doi.org/10.21203/rs.3.rs-445726/v1>.
45. USDA Agricultural Research Service. Germplasm resources information network (GRIN). USDA Agricultural Research Service Collect. 2023. <https://doi.org/10.15482/USDA.ADC/1212393>.
46. Andrews S. (2023). FastQC: a quality control tool for high throughput sequence data. Available at: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
47. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. *Bioinformatics.* 2014;30:2114–20. <https://doi.org/10.1093/bioinformatics/btu170>.
48. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics.* 2016;32:3047–8. <https://doi.org/10.1093/bioinformatics/btw354>.
49. Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol.* 2020;21:241. <https://doi.org/10.1186/s13059-020-02154-5>.

50. Adhikari B. (2025). *Morus rubra* mitogenome project. GitHub. Available at: https://github.com/abibek52/Morus_rubra_mitogenome_project
51. Tillich M, Lehwark P, Pellizzer T, Ulbricht EJS, Fischer A, Bock R, Greiner S. GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 2017;45:W6–11. <https://doi.org/10.1093/nar/gkx391>.
52. Chan PP, Lin BY, Mak AJ, Lowe TM. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res.* 2021;49:9077–96. <https://doi.org/10.1093/nar/gkab688>.
53. Kent WJ. BLAT—the BLAST-like alignment tool. *Genome Res.* 2002;12:656–64. <https://doi.org/10.1101/gr.229202>.
54. Carver T, Harris SR, Berriman M, Parkhill J, McQuillan JA. Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. *Bioinformatics.* 2011;28:464–9. <https://doi.org/10.1093/bioinformatics/btr703>.
55. Greiner S, Lehwark P, Bock R. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res.* 2019;47:W59–64. <https://doi.org/10.1093/nar/gkz238>.
56. Xiang CY, Gao F, Jakovlić I, Lei HP, Hu Y, Zhang H, Zou H, Wang GT, Zhang D. (2023). Using phylousuite for molecular phylogeny and tree-based analyses. *iMeta e87*. <https://doi.org/10.1002/imt.2.87>
57. Beier S, Thiel T, Münch T, Scholz U, Mascher M. MISA-web: a web server for microsatellite prediction. *Bioinformatics.* 2017;33:2583–5. <https://doi.org/10.1093/bioinformatics/btx198>.
58. Kurtz S, Choudhuri JV, Ohlebusch E, Schleiermacher C, Stoye J, Giegerich R. REPuter: the manifold applications of repeat analysis on a genomic scale. *Nucleic Acids Res.* 2001;29:4633–42. <https://doi.org/10.1093/nar/29.22.4633>.
59. Benson G. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.* 1999;27:573–80. <https://doi.org/10.1093/nar/27.2.573>.
60. Edera AA, Small I, Milone DH, Sanchez-Puerta MV. Deepred-Mt: deep representation learning for predicting C-to-U RNA editing in plant mitochondria. *Comput Biol Med.* 2021;136:104682. <https://doi.org/10.1016/j.combiomed.2021.104682>.
61. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215:403–10. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
62. Chen CJ, Wu Y, Li JW, Wang X, Zeng ZH, Xu J, Liu YL, Feng JT, Chen H, He YH, Xia R. TBtools-II: a one for all, all for one bioinformatics platform for biological big-data mining. *Mol Plant.* 2023;16:1733–42. <https://doi.org/10.1016/j.molp.2023.09.010>.
63. Wang Y, Tang H, Wang X, Sun Y, Joseph PV, Paterson AH. Detection of colinear blocks and synteny and evolutionary analyses based on utilization of MCScanX. *Nat Protoc.* 2024;19:2206–29. <https://doi.org/10.1038/s41596-024-00968-2>.
64. Sayers EW, Beck J, Bolton EE, Brister JR, Chan J, Comeau DC, Connor R, DiCuccio M, Farrell CM, Feldgarden M, Fine AM, Funk K, Hatcher E, Hoepfner M, Kane M, Kannan S, Katz KS, Kelly C, Klimke W, Kim S, Kimchi A, Landrum M, Lathrop S, Lu Z, Malheiro A, Marchler-Bauer A, Murphy TD, Phan L, Prasad AB, Pujar S, Sawyer A, Schmieder E, Schneider VA, Schoch CL, Sharma S, Thibaud-Nissen F, Trawick BW, Venkatapathi T, Wang J, Pruitt KD, Sherry ST. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* 2024;52:D33–43. <https://doi.org/10.1093/nar/gkad1044>.
65. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 2013;30:772–80. <https://doi.org/10.1093/molbev/mst010>.
66. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol.* 2020;37:1530–4. <https://doi.org/10.1093/molbev/msaa015>.
67. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods.* 2017;14:587–9. <https://doi.org/10.1038/nmeth.4285>.
68. Letunic I, Bork P. Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res.* 2024;52:W78–82. <https://doi.org/10.1093/nar/gkx268>.
69. Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K. Mol Biol Evol. 2024;41:msae263. <https://doi.org/10.1093/molbev/msae263>. MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing.
70. Stevens PF. (2001 onwards). Angiosperm phylogeny website. Version 14, July 2017 [and more or less continuously updated since]. Available at: <https://www.mobot.org/mobot/research/apweb/>
71. Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. *Nature.* 1981;290:457–65. <https://doi.org/10.1038/290457a0>.
72. Oda K, Yamato K, Ohta E, Nakamura Y, Takemura M, Nozato N, Akashi K, Kanegae T, Ogura Y, Kohchi T, Ohyama K. Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA: a primitive form of plant mitochondrial genome. *J Mol Biol.* 1992;223:1–7. [https://doi.org/10.1016/0022-2836\(92\)90708-R](https://doi.org/10.1016/0022-2836(92)90708-R).
73. Wang L, Liu X, Wang Y, Ming X, Qi J, Zhou Y. Comparative analysis of the mitochondrial genomes of four *Dendrobium* species (Orchidaceae) reveals heterogeneity in structure, synteny, intercellular gene transfer, and RNA editing. *Front Plant Sci.* 2024;15–2024. <https://doi.org/10.3389/fpls.2024.1429545>.
74. Gualberto JM, Newton KJ. Plant mitochondrial genomes: dynamics and mechanisms of mutation. *Annu Rev Plant Biol.* 2017;68:225–52. <https://doi.org/10.1146/annurev-arplant-043015-112232>.
75. Adams KL, Qiu YL, Stoutemyer M, Palmer JD. (2002). Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proceedings of the National Academy of Sciences* 99:9905–9912. <https://doi.org/10.1073/pnas.042694899>
76. Lin D, Shao B, Gao Z, Li J, Li Z, Li T, Huang W, Zhong X, Xu C, Chase MW, Jin X. Phylogenomics of angiosperms based on mitochondrial genes: insights into deep node relationships. *BMC Biol.* 2025;23:45. <https://doi.org/10.1186/s12915-025-02135-9>.
77. Wei L, Zheng T, Xiang J, Cheng J, Wu J. Assembly and analysis of the first complete mitochondrial genome of *Ficus carica* Linn. *J Am Soc Hortic Sci.* 2023;148:283–91. <https://doi.org/10.21273/JASHS05328-23>.
78. Qiu X, Tian Y, Li Z, Wu X, Xiang Z, Wang Y, Li J, Xiao S. Assembly and characterization analysis of the complete mitochondrial genome of *Lithocarpus litseifolius* (Hance) Chun. *Genet Resour Crop Evol.* 2025;72:295–313. <https://doi.org/10.1007/s10722-024-01989-2>.
79. Liu X, Zhang D, Yu Z, Zeng B. Assembly and analysis of the complete mitochondrial genome of the Chinese wild dwarf almond (*Prunus tenella*). *Front Genet.* 2024;14–2023. <https://doi.org/10.3389/fgenet.2023.1329060>
80. Zhang Y, Guo X, Ma R, Yu M, Xu J, Shen Z. Assembly and analysis of the complete mitochondrial genome of *Prunus davidiana* (Rosaceae). *Front Plant Sci.* 2025;16:1558619. <https://doi.org/10.3389/fpls.2025.1558619>.

81. Bi C, Lu N, Xu Y, He C, Lu Z. Characterization and analysis of the mitochondrial genome of common bean (*Phaseolus vulgaris*) by comparative genomic approaches. *Int J Mol Sci.* 2020;21:3778. <https://doi.org/10.3390/ijms21113778>.
82. Guo W, Zhu A, Fan W, Mower JP. Complete mitochondrial genomes from the ferns *Ophioglossum californicum* and *Psilotum nudum* are highly repetitive with the largest organellar introns. *New Phytol.* 2017;213:391–403. <https://doi.org/10.1111/nph.14135>.
83. Zeng Z, Zhang Z, Norbu N, Bonjor N, Tan X, Zhang S, Tso N, Wang J, Qiong L. Comprehensive analysis of the complete mitochondrial genome of *Paeonia ludlowii* reveals a dual-circular structure and extensive inter-organellar gene transfer. *Biology.* 2025;14:854. <https://doi.org/10.3390/biology14070854>.
84. Wang R, Luo Y, Lan Z, Qiu D. Insights into structure, codon usage, repeats, and RNA editing of the complete mitochondrial genome of *Perilla frutescens* (Lamiaceae). *Sci Rep.* 2024;14:13940. <https://doi.org/10.1038/s41598-024-64509-3>.
85. Cole LW, Guo W, Mower JP, Palmer JD. High and variable rates of repeat-mediated mitochondrial genome rearrangement in a genus of plants. *Mol Biol Evol.* 2018;35:2773–85. <https://doi.org/10.1093/molbev/msy176>.
86. Ke SJ, Liu DK, Tu XD, He X, Zhang MM, Zhu MJ, Zhang DY, Zhang CL, Lan SR, Liu ZJ. *Apostasia* mitochondrial genome analysis and monocot mitochondria phylogenomics. *Int J Mol Sci.* 2023;24:7837. <https://doi.org/10.3390/ijms24097837>.
87. Khara P, Saxena R, Sameerkumar C, Saxena K, Varshney RK. Mitochondrial SSRs and their utility in distinguishing wild species, CMS lines and maintainer lines in Pigeonpea (*Cajanus cajan* L.). *Euphytica.* 2015;206:737–46. <https://doi.org/10.1007/s10681-015-1504-2>.
88. Sharp PM, Li WH. An evolutionary perspective on synonymous codon usage in unicellular organisms. *J Mol Evol.* 1986;24:28–38. <https://doi.org/10.1007/BF02099948>.
89. Bi C, Paterson AH, Wang X, Xu Y, Wu D, Qu Y, Jiang A, Ye Q, Ye N. Analysis of the complete mitochondrial genome sequence of the diploid cotton *Gossypium raimondii* by comparative genomics approaches. *Biomed Res Int.* 2016;2016:5040598. <https://doi.org/10.1155/2016/5040598>.
90. Zhang J, Fu XX, Li RQ, Zhao X, Liu Y, Li MH, Zwaenepoel A, Ma H, Goffinet B, Guan YL, Xue JY, Liao YY, Wang QF, Wang QH, Wang JY, Zhang GQ, Wang ZW, Jia Y, Wang MZ, Dong SS, Yang JF, Jiao YN, Guo YL, Kong HZ, Lu AM, Yang HM, Zhang SZ, Peer YV, Liu ZJ, Chen ZD. The hornwort genome and early land plant evolution. *Nat Plants.* 2020;6:107–18. <https://doi.org/10.1038/s41477-019-0588-4>.
91. Grewe F, Edger PP, Keren I, Sultan L, Pires JC, Ostersetter-Biran O, Mower JP. Comparative analysis of 11 Brassicales mitochondrial genomes and the mitochondrial transcriptome of *Brassica oleracea*. *Mitochondrion.* 2014;19:135–43. <https://doi.org/10.1016/j.mito.2014.05.008>.
92. Hao W, Liu G, Wang W, Shen W, Zhao Y, Sun J, Yang Q, Zhang Y, Fan W, Pei S. RNA editing and its roles in plant organelles. *Front Genet.* 2021;12:757109. <https://doi.org/10.3389/fgene.2021.757109>.
93. Hu Q, Li Q, Mao Y, Luo Y, Deng Z, Zhang W. Assembly and analysis of the complete mitochondrial genome of *Leonurus japonicus* (Lamiaceae). *Sci Rep.* 2025;15:13372. <https://doi.org/10.1038/s41598-025-97594-z>.
94. Liu J, Hu JY, Li DZ. Remarkable mitochondrial genome heterogeneity in *Meniocus linifolius* (Brassicaceae). *Plant Cell Rep.* 2024;43:36. <https://doi.org/10.1007/s00299-023-03102-w>.
95. Perrin R, Meyer EH, Zaepfel M, Kim YJ, Mache R, Grienemberger JM, Gualberto JM, Gagliardi D. Two exoribonucleases act sequentially to process mature 3'-ends of *atp9* mRNAs in *Arabidopsis* mitochondria. *J Biol Chem.* 2004;279:25440–6. <https://doi.org/10.1074/jbc.M401182200>.
96. Handa H, Kubo N, Kadowaki K. Genes for the ribosomal S4 protein encoded in higher plant mitochondria are transcribed, edited, and translated. *Mol Gen Genet.* 1998;258:199–207. <https://doi.org/10.1007/s004380050723>.
97. Siqueira SF, Dias SG, Hardouin P, Pereira FR, Lejeune B, de Souza AP. Transcription of succinate dehydrogenase subunit 4 (*sdh4*) gene in potato: detection of extensive RNA editing and co-transcription with cytochrome oxidase subunit III (*cox3*) gene. *Curr Genet.* 2002;41:282–90. <https://doi.org/10.1007/s00294-002-0294-y>.
98. Varré JS, D'Agostino N, Touzet P, Gallina S, Tamburino R, Cantarella C, Ubrig E, Cardi T, Drouard L, Gualberto JM, Scotti N. Complete sequence, multichromosomal architecture and transcriptome analysis of the *Solanum tuberosum* mitochondrial genome. *Int J Mol Sci.* 2019;20:4788. <https://doi.org/10.3390/ijms20194788>.
99. Li J, Tang H, Luo H, Tang J, Zhong N, Xiao L. Complete mitochondrial genome assembly and comparison of *Camellia sinensis* var. *Assamica* cv. *Dunsa*. *Front Plant Sci.* 2023;14. <https://doi.org/10.3389/fpls.2023.1117002>.
100. Lai C, Wang J, Kan S, Zhang S, Li P, Reeve WG, Wu Z, Zhang Y. Comparative analysis of mitochondrial genomes of *Broussonetia* spp. (Moraceae) reveals heterogeneity in structure, syteny, intercellular gene transfer, and RNA editing. *Front Plant Sci.* 2022;13:1052151. <https://doi.org/10.3389/fpls.2022.1052151>.
101. Sugiyama Y, Watase Y, Nagase M, Makita N, Yagura S, Hirai A, Sugiura M. The complete nucleotide sequence and multipartite organization of the tobacco mitochondrial genome: comparative analysis of mitochondrial genomes in higher plants. *Mol Genet Genomics.* 2005;272:603–15. <https://doi.org/10.1007/s00438-004-1075-8>.
102. Yong Y, Wang Y, Wang D, Yuan X, Zhang Q. The organelle genomes of the endangered seagrass *Zostera caespitosa* reveal sequence divergences, massive gene transfer, and uncommon RNA editing types. *Front Plant Sci.* 2025;16:1550467. <https://doi.org/10.3389/fpls.2025.1550467>.
103. Tang P, Ni Y, Li J, Lu Q, Liu C, Guo J. The complete mitochondrial genome of *Paeonia lactiflora* Pall. (Saxifragales: Paeoniaceae): evidence of gene transfer from chloroplast to mitochondrial genome. *Genes.* 2024;15:239. <https://doi.org/10.3390/genes15020239>.
104. Li G, Zhang H, Lin Z, Li H, Xu G, Xu Y, Ji R, Luo W, Qiu Y, Qiu S, Tang H. Comparative analysis of chloroplast and mitochondrial genomes of sweet potato provides evidence of gene transfer. *Sci Rep.* 2024;14:4547. <https://doi.org/10.1038/s41598-024-55150-1>.
105. Wang D, Wu YW, Shih ACC, Wu CS, Wang YN, Chaw SM. Transfer of chloroplast genomic DNA to mitochondrial genome occurred at least 300 MYA. *Mol Biol Evol.* 2007;24:2040–8. <https://doi.org/10.1093/molbev/msm133>.
106. Schleicher S, Binder S. In *Arabidopsis thaliana* mitochondria 5' end polymorphisms of *nad4L-atp4* and *nad3-rps12* transcripts are linked to RNA PROCESSING factors 1 and 8. *Plant Mol Biol.* 2021;106:335–48. <https://doi.org/10.1007/s11103-021-01153-9>.
107. Huang J, Zhang D, Yang S, Liu Y, Ma J, Zhou M, Tan Y, Song Y. Genomic insights and phylogenetics of the mitochondrial genome of *Cryptocarya*. *Genomics.* 2025;117:111018. <https://doi.org/10.1016/j.ygeno.2025.111018>.

108. Liu F, Fan W, Yang JB, Xiang CL, Mower JP, Li DZ, Zhu A. Episodic and guanine–cytosine-biased bursts of intragenomic and interspecific synonymous divergence in Ajugoideae (Lamiaceae) mitogenomes. *New Phytol.* 2020;228:1107–14. <https://doi.org/10.1111/nph.16753>.
109. Palmer JD, Herbon LA. Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. *J Mol Evol.* 1988;28:87–97. <https://doi.org/10.1007/bf02143500>.
110. Duminil J, Besnard G. Utility of the mitochondrial genome in plant taxonomic studies. *Methods Mol Biol.* 2021;2222:107–18. https://doi.org/10.1007/978-1-0716-0997-2_6.
111. Govindarajulu R, Parks M, Tennessen JA, Liston A, Ashman TL. Comparison of nuclear, plastid, and mitochondrial phylogenies and the origin of wild octoploid strawberry species. *Am J Bot.* 2015;102:544–54. <https://doi.org/10.3732/ajb.1500026>.
112. Gardner EM, Garner M, Cowan R, Dodsworth S, Epitawalage N, Arifiani D, Sahromi, Baker WJ, Forest F, Maurin O, Zerega NJC, Monro AK, Hipp AL. Repeated parallel losses of inflexed stamens in Moraceae: Phylogenomics and generic revision of the tribe Moreae and the reinstatement of the tribe Olmedieae (Moraceae). *Taxon.* 2021;70:946–88. <https://doi.org/10.1002/tax.12526>.
113. Yang CX, Liu SY, Zerega NJC, Stull GW, Gardner EM, Tian Q, Gu W, Lu Q, Folk RA, Kates HR, Guralnick RP, Soltis DE, Soltis PS, Wang YH, Yi TS. Phylogeny and biogeography of *Morus* (Moraceae). *Agronomy.* 2023;13:2021. <https://doi.org/10.3390/agronomy13082021>.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.