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# Comparative mitochondrial genome analysis of Panax japonicus var. major reveals genome organization, evolutionary patterns, and phylogenetic implications

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# Abstract

*Panax japonicus* var. *major*, a medicinally important plant with ecological and pharmaceutical value, remains poorly characterized at the mitochondrial genome level. Here, we report the first complete mt genome assembly and annotation of *P. japonicus* var. *major* using a hybrid sequencing approach combining Illumina short reads and Nanopore long reads. The mt genome is 702,939 bp in length and contains 58 annotated genes, including 35 protein-coding genes (PCGs), 20 tRNA genes, and 3 rRNA genes. Comprehensive analyses of repetitive sequences revealed the presence of simple sequence repeats (SSRs), tandem repeats, and dispersed repeats, which may contribute to genome structural variation and recombination. RNA-editing prediction identified 572 putative editing sites, all involving C-to-U conversions, with several PCGs undergoing extensive post-transcriptional modification. Ka/Ks analysis showed that most mitochondrial PCGs were subject to purifying selection, whereas a small number of genes, including atp8, rps7, and rpl10, exhibited elevated evolutionary rates, suggesting lineage-specific selective pressures. In addition, 29 mitochondrial plastid-derived sequences (MTPTs), including 12 retained chloroplast-derived genes, were detected, indicating historical plastid-to-mitochondrion DNA transfer. Phylogenetic analyses based on mitochondrial PCGs resolved the evolutionary placement of *P. japonicus* var. *major* within Araliaceae and confirmed its close relationship with other *Panax* species. Collectively, this study expands mitochondrial genomic resources for *P. japonicus* var. *major* and provides a molecular foundation for investigating organelle evolution, phylogenetic relationships, germplasm identification, and conservation of this valuable medicinal plant.


# Background

*Panax japonicus* C. A. Mey. var. *major* (Burk.) C. Y. Wu et K. M. Feng (*P. japonicus* var. *major*), a notable medicinal resource within the genus *Panax* (Araliaceae), has long been used in traditional Chinese medicine due to its diverse bioactive constituents [1, 2]. Phytochemical investigations have revealed that this species contains triterpenoid saponins, polysaccharides, phenolic compounds, volatile oils, amino acids, and trace elements [3]. Among these metabolites, triterpenoid saponins represent the major pharmacologically active components and have been associated with various biological activities, including anti-inflammatory, anti-tumor, hepatoprotective, and immunomodulatory effects [4, 5]. The rhizomes of *P. japonicus* var. *major* are widely utilized as raw materials in numerous pharmaceutical and health-related products. However, increasing anthropogenic pressure and habitat specificity have raised concerns regarding the conservation status of this species. Due to restricted ecological distribution, low natural regeneration capacity, and excessive exploitation, wild populations of *P. japonicus* var. *major* have declined considerably, resulting in its recognition as an endangered taxon in the China Species Red List [6]. With the expansion of cultivation and increasing demand for medicinal resources, maintaining genetic diversity and improving germplasm utilization have become important priorities for sustainable development and conservation. Although extensive studies have focused on the chemical composition and pharmacological properties of *P. japonicus* var. *major*, genomic information for this species remains limited. The genetic architecture and evolutionary characteristics of *P. japonicus* var. *major* remain largely unresolved. Therefore, comprehensive genomic studies are needed to establish molecular resources for

understanding its evolutionary history, conserving genetic diversity, and supporting future breeding programs.

Genomic information provides critical molecular resources for germplasm evaluation and molecular breeding. In plants, mt genomes play essential roles in energy production, cellular regulation, stress adaptation, and evolutionary diversification [7]. Recent advances in sequencing technologies and bioinformatics have enabled comprehensive characterization of genomic variation and evolutionary patterns in *Panax* species [8]. However, plant mt genomes remain challenging to analyze due to their large size, structural complexity, frequent rearrangements, and incorporation of foreign DNA sequences, which complicate genome assembly and annotation [9]. For *P. japonicus* var. *major*, the absence of comprehensive mitochondrial genomic information has limited our understanding of its genetic organization, evolutionary history, and potential utilization in molecular breeding.

In this study, we assembled and annotated the mt genome of *P. japonicus* var. *major* and performed a systematic characterization of its genomic features. We investigated repetitive elements, codon usage patterns, and RNA-editing profiles of mitochondrial protein-coding genes (PCGs) to elucidate genome structure and post-transcriptional regulation. In addition, the chloroplast genome was assembled, and mitochondrial plastid-derived sequences (MTPTs) were identified to evaluate inter-organelle DNA transfer. Phylogenetic relationships were reconstructed using mitochondrial PCGs from 18 representative species, while comparative synteny analyses among nine related species were conducted to examine mt genome evolution and structural conservation. Together, these analyses provide new insights into the mt genome characteristics, evolutionary dynamics, and genetic resources of *P. japonicus* var. *major*, facilitating future studies on species identification, conservation, and molecular improvement.

## Material and methods

# Plant material sampling, DNA extraction, and sequencing

The young and healthy leaves of *P. japonicus* var. *major* were collected from privately cultivated field in Lijiang, Yunnan Province, China (coordinates: 100°02'14.56"E, 26°45'31.85"N) (Fig. 1). Plant sampling was conducted with the required permissions and complied with relevant institutional, national, and international regulations governing the collection and use of plant materials. Species identity was confirmed by Professor Aien Tao, and a voucher specimen (PY20250807) was deposited in the Herbarium of Lijiang Culture and Tourism College. Fresh leaves were pooled and finely ground prior to DNA extraction. High-molecular-weight genomic DNA was isolated using a modified CTAB protocol [10]. DNA integrity and concentration were evaluated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Qualified DNA samples were subsequently submitted to Novogene Biotech Co., Ltd. (Beijing, China) for library construction and sequencing. For long-read sequencing, genomic DNA libraries were prepared using the SQK-LSK109 ligation kit and sequenced on the Oxford Nanopore PromethION platform (Oxford Nanopore Technologies, Oxford, UK). Raw electrical signals

were converted into nucleotide sequences using the platform's standard base-calling workflow. The resulting reads were quality-filtered with NanoFilt, and sequencing quality was further evaluated using NanoPlot from the NanoPack toolkit. To improve assembly accuracy, paired-end short-read sequencing was additionally performed on the Illumina NovaSeq X Plus platform (Illumina Inc., San Diego, CA, USA). The resulting Illumina reads were processed with fastp v0.23.1 using default settings before subsequent genome assembly and polishing.

## Mitochondrial genome assembly and annotation

The chloroplast genome was assembled from Illumina short-read data using GetOrganelle with the parameters "-R 15 -k 21,45,65,85,105 -F embplant_pt" [11]. Meanwhile, the mt genome of *P. japonicus* var. *major* was assembled de novo using the autoMito workflow implemented in PMAT v2.1.0 [12]. In this workflow, polished Nanopore reads were first partitioned into smaller fragments for assembly with Newbler. Conserved mitochondrial protein-coding genes (PCGs) were subsequently used as BLASTn queries to identify mitochondrial seed contigs. These contigs were progressively connected and extended based on graph linkage information to reconstruct the mt genome. Contigs lacking mitochondrial characteristics or assembly connectivity were excluded, and the resulting assembly graph was examined using Bandage v0.8.1 [13]. The complete chloroplast genome was annotated using the CPGAVAS2 web server (http://47.96.249.172:16019/analyzer/annotate) [14]. For mt genome annotation, six published mt genomes from the Araliaceae family were selected as references, including *Panax ginseng* (MW029460.1), *Panax quinquefolius* (NC_067574.1), *Polyscias racemosa* (PP894747.1), *Polyscias bisattenuata* (PP894748.1), *Eleutherococcus senticosus* (PP894749.1), and *Polyscias macgillivrayi* (PP894750.1). The finalized mt genome assembly of *P. japonicus* var. *major* has been deposited in the NCBI GenBank database under accession number PZ790877.

## Analysis of codon usage bias, repeat fragments prediction of RNA editing sites

Simple sequence repeats (SSRs) in the mt genome were identified using MISA v2.1 [15]. The minimum repeat thresholds were set to 10 for mononucleotide repeats, 6 for dinucleotide repeats, and 5 for tri-, tetra-, penta-, and hexanucleotide repeats. Tandem repeats were subsequently detected using Tandem Repeats Finder v4.09 with the parameters 2 7 7 80 10 50 500 -f -d -m [16]. Dispersed repeats were further identified using vmatch v2.3.1, with a minimum repeat length of 30 bp and a maximum Hamming distance of 3. Protein-coding gene (PCG) sequences were extracted from the annotated mt genome using PhyloSuite v1.2.2 [17]. Codon usage frequency and relative synonymous codon usage (RSCU) values were then calculated with CodonW, and the resulting data were visualized using custom R scripts based on the ggplot package [18]. Putative RNA editing sites within mitochondrial PCGs were predicted using Deepred-Mt v0.1.0, with only predictions having a confidence probability of ≥ 0.90 retained for subsequent analyses [19].

## Identification of homologous fragments and collinear analysis

Mitochondrial plastid DNA (MTPT) sequences represent plastid-derived DNA fragments integrated into the mt genome and may contribute to the transfer of functional genetic elements, including codons associated with protein-coding genes, as well as influence post-transcriptional RNA processing [20, 21]. To characterize MTPTs in *P. japonicus* var. *major*, homologous regions between the chloroplast and mt genomes were identified using BLASTn with the following parameters: -evalue 1e-5, -word_size 9, -gapopen 5, -gapextend 2, -reward 2, and -penalty – 3. The identified homologous fragments were subsequently annotated using GeSeq, and their genomic relationships were visualized with Circos v0.69-5 [22]. To avoid redundant counting, MTPTs located within the inverted repeat (IR) regions of the chloroplast genome were recorded only once. To further evaluate genome conservation and structural collinearity, syntenic relationships between the mt genome of *P. japonicus* var. *major* and those of closely related species were analyzed using MCScanX, and the resulting multiple synteny plots were generated accordingly [23].

## Analysis of non-synonymous (Ka) and synonymous (Ks) mutation rates

To assess the selective pressures exerted on mitochondrial genes, we employed Ka/Ks_Calculator v2.0 (https://sourceforge.net/projects/kakscalculator2) to calculate the ratio of non-synonymous substitution rate (Ka) to synonymous substitution rate (Ks) for PCGs common to the species depicted in the *P. japonicus* var. *major* and others 8 species (*Panax ginseng*, *Panax quinquefolius*, *Polyscias racemosa*, *Polyscias bisattenuata*, *Eleutherococcus senticosus*, *Polyscias macgillivrayi*, *Cnidium monnieri*, *Oenanthe hookeri*). The resulting Ka/Ks values were subsequently summarized and visualized as box plots using Origin 2021 to compare the distribution of selective pressures among different mitochondrial genes.

# Phylogenetic analyses

To investigate the phylogenetic position of *P. japonicus* var. *major*, complete mt genome sequences from 18 species representing six families (Araliaceae, Apiaceae, Caprifoliaceae, Menyanthaceae, Scrophulariaceae, and Ericaceae) were retrieved from the National Center for Biotechnology Information (NCBI) database. Orthologous mitochondrial PCGs among these species were aligned using MAFFT [24]. The resulting alignments were further refined with trimAl v1.4.rev15 using the parameter "-gt 0.7" to remove poorly aligned regions and improve alignment quality. Based on the optimized sequence dataset, a maximum-likelihood (ML) phylogenetic tree was reconstructed using IQ-TREE v2.2.0 with the ModelFinder Plus option (-m MFP) and 1,000 bootstrap replicates to evaluate branch support [25].

## Results

**Features of the Panax japonicus var. major mt genome**

The accuracy and completeness of the mt genome assembly were supported by the strong agreement between Nanopore long-read and Illumina short-read sequencing data. In addition, assembly graph inspection using Bandage verified the connectivity of all contigs and confirmed the integrity of the final circular genome assembly. As illustrated in Fig. 2, the mt genome of *P. japonicus* var. *major* consists of a single circular chromosome spanning 702,939 bp with an overall GC content of 45.20%. The nucleotide composition was slightly biased toward A and T bases, with an A + T content of 54.8%, comprising A (27.4%), T (27.4%), G (22.7%), and C (22.5%) (Supplementary Table S1).

Genome annotation identified 58 unique functional genes, including 35 PCGs, 20 transfer RNA (tRNA) genes, and 3 ribosomal RNA (rRNA) genes (Table 1; Fig. 3). Gene copy number analysis further revealed that eight genes were present in multiple copies. Among them, *trnM-CAT* exhibited the highest copy number, with seven copies, whereas *rrn26* and several tRNA genes, including *trnC-GCA* and trnE-TTC, each occurred in two copies. These duplicated genes indicate that gene duplication is a common feature of the mt genome of *P. japonicus* var. *major*. Gene structure analysis revealed that nine genes (*ccmFc*, *nad1*, *nad2*, etc.) possess a multi-exon structure. Notably, the 4 exons of the *nad5* gene are distributed at distant locations, making it the most dispersed gene in the genome.

Table 1
Gene composition in the mt genome of *P. japonicus* var. *major*.

| Group of genes | | Name of genes |
|---|---|---|
| Core genes | ATP synthase | *atp1*, *atp4*, *atp6*, *atp8*, *atp9* |
| | Cytochrome c biogenesis | *ccmB*, *ccmC*, *ccmFC**, *ccmFN* |
| | Ubichinol cytochrome c reductase | *cob* |
| | Cytochrome c oxidase | *cox1*, *cox2*, cox3* |
| | NADH dehydrogenase | *nad1**, *nad2**, *nad3*, *nad4**, *nad4L*, *nad5**, *nad6*, *nad7**, *nad9* |
| | Transport membrane protein | *mttB, matR* |
| Variable genes | Ribosomal protein large subunit | *rpl10*, *rpl16* |
| | Ribosomal protein small subunit | *rps1*, *rps3**, *rps4*, *rps7*, *rps12*, *rps13*, *rps14* |
| | Succinate dehydrogenase | *sdh3*, *sdh4* |
| rRNA | Ribosome RNA | *rrn18*, *rrn26*$^{(2)}$, *rrn5* |
| tRNA | Transfer RNA | *trnC-GCA*$^{(2)}$, *trnD-GTC*$^{(2)}$, *trnE-TTC*$^{(2)}$, *trnF-AAA**, *trnF-GAA*, *trnG-GCC*, *trnH-GTG*, *trnK-CTT*, *trnK-TTT*, *trnL-TAA*, *trnM-CAT*$^{(7)}$, *trnN-GTT*, *trnP-TGG*, *trnQ-TTG*$^{(2)}$, *trnS-GCT*, *trnS-GGA*, *trnS-TGA*$^{(2)}$, *trnT-GGT*, *trnW-CCA*, *trnY-GTA*$^{(2)}$ |

Notes: *: intron; Gene (2): Number of copies of multi-copy genes;

# Analysis of codon usage bias

Codon usage patterns of the mitochondrial protein-coding genes (PCGs) were characterized by calculating the relative synonymous codon usage (RSCU) values. A total of 64 codons encoding 20 amino acids were identified (Fig. 4). Among them, leucine (Leu), serine (Ser), and arginine (Arg) were the most frequently represented amino acids, accounting for 10.29%, 9.37%, and 6.93% of all codons, respectively, whereas cysteine (Cys) was the least abundant, representing only 1.35%. Analysis of codon preference revealed that 31 codons had RSCU values ≥ 1, indicating preferential usage during protein translation. Of these preferred codons, 27 ended with A or T, three (TTG, ATG, and TGG) terminated with

G, and only one (ACC) ended with C, demonstrating a pronounced preference for A/T-ending synonymous codons in the mt genome. As summarized in Table 2, the alanine codon GCT exhibited the strongest usage bias, with the highest RSCU value (1.605). Relatively high codon preferences were also observed for TAT (tyrosine; RSCU = 1.546) and CAA (glutamine; RSCU = 1.507), suggesting unequal usage among synonymous codons for these amino acids. In addition to codon usage bias, variation was observed in translation initiation codons. Although ATG was the predominant initiation codon, several genes utilized alternative start codons, including GTG in *atp1* (generated by a T-to-A substitution) and ACG in *cox1* and *nad4L* (converted to AUG through C-to-U RNA editing), highlighting the diversity of translation initiation signals in the mt genome.

Table 2
Relative synonymous codon usage for each amino acid in the mt genome of *P. japonicus* var. *major*.

| Amino acid | codon | count | RSCU | Amino acid | codon | count | RSCU |
|---|---|---|---|---|---|---|---|
| Ala | GCG | 80 | 0.502 | Leu | TTG | 203 | 1.186 |
| Ala | GCC | 151 | 0.947 | Leu | TTA | 231 | 1.35 |
| Ala | GCA | 151 | 0.947 | Leu | CTT | 233 | 1.361 |
| Ala | GCT | 256 | 1.605 | Lys | AAG | 168 | 0.798 |
| Arg | CGC | 71 | 0.616 | Lys | AAA | 253 | 1.202 |
| Arg | AGG | 83 | 0.72 | Met | ATG | 269 | 1 |
| Arg | CGG | 88 | 0.763 | Phe | TTC | 279 | 0.875 |
| Arg | CGT | 145 | 1.257 | Phe | TTT | 359 | 1.125 |
| Arg | CGA | 148 | 1.283 | Pro | CCG | 93 | 0.644 |
| Arg | AGA | 157 | 1.361 | Pro | CCC | 114 | 0.789 |
| Asn | AAC | 101 | 0.652 | Pro | CCA | 173 | 1.197 |
| Asn | AAT | 209 | 1.348 | Pro | CCT | 198 | 1.37 |
| Asp | GAC | 103 | 0.644 | Ser | AGC | 97 | 0.622 |
| Asp | GAT | 217 | 1.356 | Ser | TCG | 126 | 0.809 |
| Cys | TGC | 49 | 0.726 | Ser | TCC | 151 | 0.969 |
| Cys | TGT | 86 | 1.274 | Ser | AGT | 160 | 1.027 |
| Gln | CAG | 68 | 0.493 | Ser | TCA | 186 | 1.194 |
| Gln | CAA | 208 | 1.507 | Ser | TCT | 215 | 1.38 |
| Glu | GAG | 133 | 0.66 | Thr | ACG | 74 | 0.58 |
| Glu | GAA | 270 | 1.34 | Thr | ACA | 125 | 0.98 |
| Gly | GGC | 93 | 0.535 | Thr | ACC | 136 | 1.067 |
| Gly | GGG | 126 | 0.725 | Thr | ACT | 175 | 1.373 |
| Gly | GGT | 224 | 1.289 | Trp | TGG | 144 | 1 |
| Gly | GGA | 252 | 1.45 | Tyr | TAC | 72 | 0.454 |
| His | CAC | 62 | 0.494 | Tyr | TAT | 245 | 1.546 |
| His | CAT | 189 | 1.506 | Val | GTC | 119 | 0.759 |

| Amino acid | codon | count | RSCU | Amino acid | codon | count | RSCU |
|---|---|---|---|---|---|---|---|
| Ile | ATC | 208 | 0.82 | Val | GTG | 137 | 0.874 |
| Ile | ATA | 215 | 0.848 | Val | GTT | 184 | 1.174 |
| Ile | ATT | 338 | 1.332 | Val | GTA | 187 | 1.193 |
| Leu | CTG | 86 | 0.502 | Stop | TAG | 7 | 0.656 |
| Leu | CTC | 115 | 0.672 | Stop | TGA | 8 | 0.75 |
| Leu | CTA | 159 | 0.929 | Stop | TAA | 17 | 1.594 |

# Analysis of repeat fragments

Repetitive DNA is a major contributor to the structural complexity and size variation of plant mt genomes, with dispersed repeats, simple sequence repeats (SSRs), and tandem repeats representing the predominant repeat types. Owing to their abundance and involvement in genome recombination and structural rearrangements, these repetitive elements have long been recognized as key features of angiosperm mt genomes. In the mt genome of *P. japonicus* var. *major*, 1,987 dispersed repeats (> 30 bp) were identified, spanning 145,446 bp, which accounted for 20.69% of the total genome length. Among these repeats, 1,047 were classified as forward repeats, whereas 940 were palindromic repeats. No reverse or complementary repeats were detected. The repeat size distribution was strongly biased toward short sequences. Specifically, 73.68% of the dispersed repeats ranged from 30 to 49 bp, whereas only 3.32% exceeded 100 bp in length. Notably, 20 repeat pairs were longer than 1,000 bp (Supplementary Table S2), indicating the presence of a small number of large repeated elements that may contribute to mt genome structural variation.

A total of 32 simple sequence repeats (SSRs) were identified in the mt genome of *P. japonicus* var. *major* (Fig. 5). Mononucleotide repeats represented the largest SSR class (53.13%), followed by dinucleotide (28.13%), trinucleotide (12.50%), and pentanucleotide repeats (6.25%). No tetra- or hexanucleotide SSRs were detected (Supplementary Table S3). Among the mononucleotide SSRs, A/T motifs predominated, whereas C/G mononucleotide repeats were absent. For dinucleotide SSRs, AT/TA and AG/CT motifs accounted for 55.56% and 44.44% of the total, respectively. To further compare SSR abundance among related taxa, mt genomes from nine species within Apiales (*Cnidium monnieri*, *Panax ginseng*, *P. japonicus* var. *major*, *Panax quinquefolius*, *Polyscias bisattenuata*, *Polyscias macgillivrayi*, *Polyscias racemosa*, *Eleutherococcus senticosus*, and *Oenanthe hookeri*) were analyzed. The number of SSRs varied from 14 to 32 across these species (Supplementary Table S4).

In addition to SSRs, tandem repeats were also characterized. A total of 21 tandem repeat loci, ranging from 5 to 39 bp in length, were detected in the mt genome of *P. japonicus* var. *major* (Supplementary Table S5). These tandem repeats represent another important class of repetitive elements that may contribute to mt genome organization and structural variation.

# Analysis of RNA editing sites

RNA editing is a post-transcriptional process that alters RNA sequences through nucleotide substitutions, insertions, or deletions, thereby increasing transcriptomic diversity and potentially affecting gene function [26]. To characterize RNA editing in the mt genome of *P. japonicus* var. *major*, putative editing sites were predicted across all 35 PCGs. A total of 572 potential RNA editing sites were identified, and all predicted events corresponded to C-to-U conversions (Supplementary Table S6; Fig. 6). The distribution of editing sites varied considerably among genes. *nad4* contained the largest number of predicted editing sites (48), followed by *ccmB* (40). Several additional genes, including *nad2*, *ccmC*, *ccmFn*, and *nad7*, each possessed more than 30 predicted editing sites. In contrast, RNA editing was rare in several ribosomal protein genes. Genes such as *rps1*, *rps7* and *rps14* exhibited fewer predicted editing events, only one sites, indicating substantial variation in RNA editing frequency among mitochondrial PCGs.

# Ka/Ks analysis

The ratio of nonsynonymous (Ka) to synonymous (Ks) substitution rates is widely used to evaluate the evolutionary forces acting on PCGs [27]. The Ka/Ks ratio facilitates the determination of whether a PCG is subject to selective pressure during the evolutionary process. When Ka/Ks < 1, it indicates that the gene is under purifying selection or stabilizing selection (suppressing the accumulation of variations); Ka/Ks > 1 reflects positive selection selection (promoting adaptive variations); and a ratio of exactly 1 conforms to the neutral evolution model. It is important to note that the Ka/Ks ratio can significantly exceed 1 only when there are significant beneficial mutations at the gene locus [28].

The distribution of Ka/Ks ratios for mitochondrial PCGs shared between *P. japonicus* var. *major* and eight other Apiales species is presented in Fig. 7. Overall, 22 genes exhibited median Ka/Ks values well below 1, indicating strong sequence conservation. Representative genes, including *nad4L*, *rps12*, *atp9*, and *nad2*, all showed consistently low Ka/Ks values. In contrast, three genes (*rpl10*, *rps7*, and *atp8*) displayed median Ka/Ks values greater than 1. An additional 10 genes, including *atp4*, *ccmC*, *ccmFc*, and *cob*, showed intermediate Ka/Ks values ranging from 0.5 to < 1. Although most genes remained under purifying selection, several genes (e.g., *cox2*, *nad5*, *rps3*, and *rps4*) exhibited occasional high-value outliers among pairwise comparisons, indicating variation in evolutionary rates across different lineages. Overall, the heterogeneous distribution of Ka/Ks values suggests distinct evolutionary patterns among mitochondrial PCGs in *P. japonicus* var. major.

# Identification of the mitochondrial plastid sequences (MTPTs)

Mitochondrial plastid DNA sequences (MTPTs) are chloroplast-derived DNA fragments that have been transferred into the mt genome through intracellular DNA exchange between organelles [29]. To investigate plastid-derived sequences in the mt genome of *P. japonicus* var. *major*, homologous regions

between the chloroplast and mt genomes were identified. A total of 29 MTPT fragments were detected, spanning 26,389 bp and accounting for 3.75% of the mt genome (Fig. 8; Supplementary Table S7). These transferred fragments contained 12 annotated genes, including 10 transfer RNA (tRNA) genes (*trnW-CCA*, *trnN-GTT*, *trnM-CAT*, *trnS-GGA*, *trnD-GTC*, *trnY-GTA*, *trnE-TTC*, *trnT-GGT*, *trnS-TGA*, *trnH-GTG*), one rRNA gene (*rrn18*), and one cytochrome c biogenesis gene (*ccmC*). The predominance of tRNA genes among the transferred sequences suggests that plastid-to-mitochondrion DNA transfer has contributed primarily to the incorporation of RNA genes into the mt genome, whereas the transfer of protein-coding genes appears to have been relatively limited.

# Phylogenetic analysis

To further clarify the phylogenetic position of *P. japonicus* var. *major*, a dataset consisting of 32 core mitochondrial protein-coding genes (PCGs) present in more than 80% of the analyzed species (e.g., *atp1*, *atp4*, *atp6*, and *atp8*) was selected for phylogenetic reconstruction. The concatenated sequences of these orthologous genes from 18 representative species were analyzed using the maximum-likelihood (ML) method. Detailed information for all species included in the analysis is provided in Supplementary Table S8. The resulting phylogenetic tree (Fig. 9) was well resolved, with strong bootstrap support across most internal nodes, indicating the robustness of the inferred evolutionary relationships.

The topology clearly separated the sampled taxa into distinct clades corresponding to their respective families and orders, demonstrating the robustness of mt genome data for phylogenetic inference. *Panax japonicus* var. *major* was nested within the Araliaceae clade and formed a strongly supported monophyletic group with *P. quinquefolius* and *P. ginseng* (bootstrap = 100). This *Panax* lineage was further clustered with *Eleutherococcus senticosus* and species of *Polyscias*, consistent with their taxonomic placement within Araliaceae. Outside Araliaceae, species representing Apiaceae, Caprifoliaceae, Menyanthaceae, Scrophulariaceae, and Ericaceae each formed distinct family-level clades with high bootstrap values, reflecting clear phylogenetic differentiation among major lineages. The overall tree topology was highly congruent with the currently accepted angiosperm phylogeny group classification, supporting the reliability of mt genome sequences for resolving interspecific relationships. These results further confirm the close evolutionary affinity among *Panax* species and provide a robust phylogenetic framework for investigating the evolution and diversification of *Panax japonicus* var. *major*.

# Collinear analysis

Collinear analysis of the mt genomes revealed extensive genome rearrangements among the nine examined species (Fig. 10). Numerous homologous blocks connected the mt genomes, indicating widespread conservation of shared genomic regions despite substantial variation in gene order and genome organization. *P. japonicus* var. *major* exhibited a high degree of sequence homology with other Araliaceae species, particularly *P. ginseng* and *P. quinquefolius*, as evidenced by the large number of homologous collinear blocks spanning their mt genomes. Nevertheless, the extensive crossing of collinear links suggests that frequent genomic rearrangements, including inversions and translocations,

have occurred during the evolution of *Panax* species. Homologous regions were also detected between *P. japonicus* var. *major* and other Araliaceae members, including *Eleutherococcus senticosus* and *Polyscias* species, although these genomes displayed a greater degree of structural divergence. In contrast, comparatively fewer conserved blocks were observed between *Panax japonicus* var. *major* and the Apiaceae species (*Cnidium monnieri* and *Oenanthe hookeri*), reflecting increased evolutionary divergence between families. Overall, these results indicate that mt genomes within Araliaceae retain substantial sequence conservation while exhibiting pronounced structural rearrangements, highlighting the dynamic evolutionary nature of plant mt genomes.

# Discussion

Mitochondria are the primary organelles responsible for cellular respiration and ATP production, thereby supplying the energy required for plant growth, development, and other essential biological processes. Compared with animal mitochondria, plant mt genomes exhibit remarkable diversity in genome size, structure, and sequence organization, reflecting their dynamic evolutionary history [30]. Despite increasing interest in plant mitochondrial genomics, genomic resources for the Araliaceae family, particularly the genus *Panax*, remain relatively limited. Recent advances in long-read sequencing technologies, together with the development of specialized assembly pipelines for plant mt genomes, have substantially improved the accuracy and completeness of mt genome assembly [11]. In the present study, although experimental validation of genome architecture (e.g., PCR amplification across putative junction regions) was not performed, the high concordance between Nanopore and Illumina sequencing data, together with assembly graph inspection, provides strong evidence supporting the accuracy and structural integrity of the assembled mt genome. As an important medicinal species within Araliaceae, *P. japonicus* var. *major* possesses considerable pharmaceutical and genetic resource value. A high-quality mt genome provides an important genomic resource for future studies of mitochondrial evolution, population genetics, germplasm identification, and molecular breeding of this species.

In the present study, we report the first complete mt genome of *P. japonicus* var. *major*. The genome consists of a single circular chromosome of 702,939 bp with an overall GC content of 45.20%. Genome annotation identified 58 unique genes, including 35 PCGs, 20 tRNA genes, and 3 rRNA genes. Comparison with previously reported mt genomes of Araliaceae species revealed that gene composition is highly conserved despite considerable variation in genome size [8, 31]. Although mt genome lengths differ substantially among species, the number and types of annotated genes remain largely stable. This pattern suggests that lineage-specific differences in mt genome size and architecture are predominantly attributable to the expansion, contraction, and rearrangement of non-coding regions rather than extensive changes in gene content.

Codon usage bias represents a fundamental evolutionary feature of genomes and can influence amino acid composition, protein synthesis efficiency, and the functional characteristics of encoded proteins [32]. In the mt genome of *P. japonicus* var. *major*, codon usage analysis revealed a pronounced preference for A/T nucleotides, particularly at the third codon position, resulting in a strong bias toward

A/T-ending codons. This pattern is consistent with the codon usage characteristics commonly observed in dicotyledonous plant mt genomes, suggesting a conserved evolutionary tendency toward A/T enrichment among plant mitochondrial genes. A total of 31 codons exhibited relative synonymous codon usage (RSCU) values greater than 1, and most of these preferentially terminated with A or T. The predominance of A/T-ending codons may reflect the combined effects of nucleotide composition constraints, mutational bias, and selective forces acting on mt genome evolution. Additionally, variations in codon preference may be associated with differences in tRNA availability and translational efficiency, thereby contributing to the regulation of mitochondrial gene expression [33]. These findings provide insights into the evolutionary characteristics of codon usage in the mt genome of *P. japonicus* var. *major*. Furthermore, understanding codon preference and RNA editing patterns may provide valuable information for future studies on mitochondrial gene regulation, germplasm evaluation, and molecular breeding strategies.

Repetitive sequences are major contributors to the dynamic evolution of plant mt genomes, influencing genome expansion, structural rearrangement, and recombination activity [30]. Among these repetitive elements, dispersed repeats are particularly important because they provide potential substrates for homologous recombination and may facilitate mt genome restructuring, including the generation of complex genome configurations [34]. In the mt genome of *P. japonicus* var. *major*, dispersed repeats were predominantly composed of short repeat sequences, with fragments ranging from 30 to 69 bp accounting for 91.19% of the identified dispersed repeats. Furthermore, 32 simple sequence repeats (SSRs) were detected, including 17 mononucleotide repeats (53.13%), 9 dinucleotide repeats (28.13%), 4 trinucleotide repeats (12.50%), and 2 pentanucleotide repeats (6.25%). No tetra- or hexanucleotide repeat motifs were identified. The composition and abundance of SSRs varied among Araliaceae species, suggesting lineage-specific differences in the accumulation and evolution of repetitive elements. For example, compared with *Eleutherococcus senticosus* (124 SSRs) [35], *P. japonicus* var. *major* contained fewer repetitive elements, which may contribute to its relatively smaller mt genome size within the family. These findings highlight the heterogeneous distribution of repetitive sequences among Araliaceae mt genomes and provide insights into their potential roles in shaping mt genome evolution.

RNA editing is a widespread and evolutionarily conserved post-transcriptional regulatory mechanism in angiosperm mitochondria. Through enzyme-mediated nucleotide modifications, primarily C-to-U conversions, RNA editing alters transcript sequences and can affect the amino acid composition and functional properties of encoded proteins. This process plays important roles in mitochondrial gene regulation, plant development, environmental adaptation, and the regulation of cytoplasmic male sterility [36]. In the mt genome of *P. japonicus* var. *major*, a total of 572 putative RNA editing sites were predicted across all annotated PCGs. The number of predicted editing events was comparable to that reported for other Araliaceae species, such as *Eleutherococcus senticosus* (587 sites) [35]. All identified editing events involved C-to-U conversions, consistent with the predominant RNA editing pattern observed in most plant mt genomes [37]. The distribution of RNA editing sites varied considerably among mitochondrial genes. The nad4 gene exhibited the highest number of predicted editing sites (48), indicating extensive post-transcriptional modification, whereas *rps1*, *rps14*, and *rps7* contained only one

editing site each, suggesting relatively limited editing activity in these genes. Such gene-specific variation may reflect differences in functional importance, evolutionary constraints, and transcript regulation among mitochondrial genes. Previous studies have shown that RNA editing can generate amino acid substitutions that increase protein stability or restore conserved amino acid residues, potentially compensating for deleterious nucleotide changes accumulated during mt genome evolution [38]. Overall, the extensive and uneven distribution of RNA editing sites in *P. japonicus* var. *major* highlights the complexity of mitochondrial post-transcriptional regulation and provides valuable insights into the evolutionary maintenance of mitochondrial gene function in Araliaceae.

The predominance of Ka/Ks values below 1 indicates that purifying selection has been the major evolutionary force shaping the mt genome of *P. japonicus* var. *major*, consistent with the highly conserved functions of mitochondrial genes in oxidative phosphorylation, ATP synthesis, and cellular respiration. Genes such as *nad4L*, *nad2*, *atp9*, and r*ps12* encode essential components of the respiratory electron transport chain, ATP synthase, or mitochondrial translation machinery, explaining their strong evolutionary conservation. In contrast, the relatively elevated Ka/Ks values observed for *rpl10*, *rps7*, and *atp8* suggest reduced selective constraints or possible lineage-specific adaptive evolution. These genes are associated with mitochondrial ribosome function or energy metabolism and may contribute to physiological adaptation related to redox homeostasis, environmental stress responses, or species diversification [39, 40]. Similarly, genes with intermediate Ka/Ks values (0.5 to < 1), such as *atp4*, *ccmC*, *ccmFc*, and *cob*, may be undergoing relatively relaxed purifying selection or gradual functional divergence rather than experiencing strong positive selection [41]. Nevertheless, Ka/Ks values approaching or exceeding 1 should be interpreted with caution. Such estimates may reflect relaxed purifying selection rather than genuine positive selection and can also be influenced by limited sequence divergence, alignment uncertainty, or substitution-rate heterogeneity among lineages [42]. Taken together, the gene-specific variation in Ka/Ks values highlights heterogeneous evolutionary constraints across mitochondrial PCGs and provides new insights into the molecular evolution of the *P. japonicus* var. *major* mt genome, while also identifying candidate genes for future studies of adaptive evolution, germplasm characterization, and phylogenetic inference.

Inter-organellar DNA transfer between chloroplasts and mitochondria represents an important evolutionary process that contributes to the dynamic remodeling of plant mt genomes. In the mt genome of *P. japonicus* var. *major*, 29 mitochondrial plastid-derived sequences (MTPTs) were identified, ranging from 30 to 2,700 bp in length. Collectively, these fragments covered 26,389 bp, accounting for 3.75% of the total mt genome. The proportion of MTPTs in *P. japonicus* var. *major* was comparable to that reported for several other plant species, including *Panax notoginseng* (3.11%) [43], *Eryngium foetidum* (2.83%) [37], and *Eleutherococcus senticosus* (1.69%) [35], but was lower than values reported for *Spuriopimpinella brachycarpa* (8.13%) [44], *Rehmannia chingii* (16.34%) [45], and *Pleione yunnanensis* (14.08%) [46]. These differences indicate considerable variation in the extent of chloroplast-derived DNA incorporation among plant mt genomes. MTPTs reflect historical plastid-to-mitochondrion DNA transfer and may contribute to mt genome evolution through sequence integration and modification [47]. Annotation of the identified MTPT regions revealed 12 intact chloroplast-derived genes, including 10 tRNA genes (*trnW-CCA*, *trnN-*

*GTT*, *trnM-CAT*, *trnS-GGA*, *trnD-GTC*, *trnY-GTA*, *trnE-TTC*, *trnT-GGT*, *trnS-TGA*, *trnH-GTG*), 1 was rRNA gene (*rrn18*), and 1 cytochrome c biogenesis gene (*ccmc*). These retained chloroplast-derived genes highlight the contribution of MTPT integration to mt genome complexity and evolution in *Panax*.

To investigate the evolutionary placement of *P. japonicus* var. *major*, a maximum likelihood (ML) phylogenetic analysis was performed using mt genome sequences from 18 representative species spanning five orders, including Apiales, Dipsacales, Asterales, Lamiales, and Ericales. The resulting ML tree showed high bootstrap values across most branches, providing strong resolution of the evolutionary relationships among the analyzed taxa. Consistent with current taxonomic classifications, *P. japonicus* var. *major* clustered within the Araliaceae lineage and formed a well-supported monophyletic clade with *Panax quinquefolius* and *Panax ginseng* (bootstrap = 100), confirming their close evolutionary relationship. This topology agrees with previous phylogenetic inferences based on chloroplast genomes reported by Yang et al. [48]. Furthermore, the *Panax* clade was grouped with *Eleutherococcus senticosus* and *Polyscias* species, supporting their shared evolutionary history within Araliaceae. Outside Araliaceae, species belonging to Apiaceae, Caprifoliaceae, Menyanthaceae, Scrophulariaceae, and Ericaceae formed distinct family-level clusters, indicating consistent phylogenetic patterns among major angiosperm lineages. Overall, mt genome data provide additional evidence for resolving relationships within Araliaceae and offer a valuable genomic framework for studying the diversification of *Panax* species.

Overall, this study identified several potential molecular resources, including SSR loci and highly variable mitochondrial regions, which may serve as useful markers for germplasm discrimination and population genetic studies. In addition, mitochondrial genes from the *nad*, *atp*, and *ccm* families represent promising candidates for investigating the evolutionary dynamics of energy metabolism and stress-related adaptation. Phylogenetic analyses based on mt genome sequences provided additional insights into the evolutionary relationships of *P. japonicus* var. *major*, confirming its close relationship with other *Panax* species and supporting its taxonomic placement within Araliaceae. Collectively, these genomic resources expand the molecular foundation for species identification, evolutionary studies, and conservation strategies of *Panax* germplasm.

## Conclusions

In this study, we generated a complete mt genome resource for *P. japonicus* var. *major* through genome assembly and annotation. The assembled mt genome exhibited a circular structure of 702,939 bp and contained 58 annotated genes, including 35 protein-coding genes, 20 tRNA genes, and 3 rRNA genes. Comparative analyses of codon usage, repetitive elements, chloroplast-derived sequences, and RNA editing patterns revealed the structural characteristics and evolutionary features of the mt genome in *P. japonicus* var. *major*. Together with phylogenetic analyses, these findings expand our understanding of mt genome evolution within *Panax* and provide molecular resources for species identification, germplasm evaluation, and conservation studies. Future investigations integrating mitochondrial variation with population-level genomic data will further elucidate the genetic mechanisms underlying adaptation and facilitate the utilization of valuable *Panax* germplasm.

## Declarations

# Clinical trial number

Not applicable

# Ethics approval and consent to participate

The collections of *P. japonicus* var. *major* were conducted with the necessary permissions. Our experimental research, including the collection of plant materials, complies with institutional, national, or international guidelines.

# Consent for publication

Not applicable.

# Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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# Author Contribution

ZJM, ZY, ZZM, ZFY, QSW, YWH, CC, and TAE participated in the conception and design of the research. ZJM and ZY analyzed and annotated the mt genome data. ZZM, ZFY, QSW, and YWH collected the species. ZJM was responsible for analyzing and processing data and writing the manuscript. CC and TAE revised the paper. All authors agreed to the submitted version of the manuscript.

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# Data Availability

The data provided in the study are submitted to the NCBI.

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