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Mitochondrial genomes of four *Dendrobium* species (Orchidaceae): a comprehensive analysis of structural diversity, synteny, and plastid-derived sequences

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Abstract

Mitochondrial genomes (mitogenomes) in plants exhibit substantial structural diversity, offering insights into evolutionary processes such as intracellular gene transfer (IGT), gene loss and genomic rearrangements. In this study, we present a detailed comparative analysis of the mitogenomes of four *Dendrobium* species (*D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum*), which are from the highly diverse Orchidaceae family. Using high-quality read data, including Illumina, PacBio and HiFi reads, we assembled and annotated their complete mitogenomes. Our findings reveal notable variability in mitogenome size (ranging from 485,249 to 773,276 bp), gene content (including differences in the number of protein-coding genes and tRNAs) and structural organization (such as conserved syntenic blocks and rearranged regions), reflecting the dynamic evolution of mitogenomes among these *Dendrobium* species. Notably, we observed high levels of simple sequence repeats (SSRs), with variations in repeat content across species, reflecting the dynamic nature of these genomes. Synteny analysis revealed extensive conserved genomic regions between the mitogenomes of *D. lasianthera* and *D. tangerinum*, indicating strong structural similarity. In contrast, other species pairs such as *D. lasianthera* and *D. huoshanense*, or *D. tangerinum* and *D. chrysotoxum*, exhibited weaker syntenic relationships, reflecting a greater degree of structural variation in their mitogenomes. Furthermore, our analysis identified IGT between mitogenome and plastome, providing clear evidence that IGT has contributed to the mitogenome evolution of these species. Phylogenetic analysis based on mitogenome coding sequences (CDSs) revealed a well-supported topology within *Dendrobium*, identifying *D. lasianthera* and *D. tangerinum* as closely related sister species. Although our sampling is limited to four *Dendrobium* species, the mitogenome CDS data still provide informative phylogenetic signals and offer complementary insights when interpreted alongside plastid-based phylogenies. This study provides new insights into the structural and genomic diversity of mitogenomes in *Dendrobium* and contributes to the broader understanding of mitogenome evolution in plants.

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Introduction

Mitochondria, often referred to as the energy powerhouses of eukaryotic cells, contain their own genome, which encodes essential genes for cellular respiration and energy production[1]. In plants, mitogenomes are typically large, complex, and highly variable, often exhibiting a diverse range of structural features across different species[2,3]. Despite the simplicity associated with single parental inheritance, ongoing processes such as intracellular gene transfers (IGTs), genome reduction and rearrangement, collectively influence mitogenomic diversity[4]. There is significant variability in plant mitogenomes, not only between distantly related species but also within closely related groups, such as at the family or genus level[5]. The orchid family (Orchidaceae) is one of the most species-rich plant families, with remarkable diversity in both morphological and genetic traits[6]. Among the many orchid genera, *Dendrobium* is a widely distributed group that includes over 1,800 species found across diverse ecological niches[7]. Due to their economic, ecological and ornamental importance, *Dendrobium* species have become the subject of extensive genetic studies, yet the characterization of their mitogenomes remains limited[8]. Recent advances in next-generation sequencing (NGS) and third-generation sequencing technologies have made it possible to obtain high-quality mitogenomes, revealing extensive structural variation, gene content differences and potential IGT[9,10].

Mitogenome structural conformations exhibit a wide diversity of architectures often mediated by repetitive sequences[11] or the insertion of foreign sequences[12]. Furthermore, intracellular DNA transfer among plastid, mitochondrial and nuclear genomes led to the existence of derived fragments, and promotes genome evolution[13].

Mitogenomes in orchids are known for their significant structural diversity and complexity. For *Dendrobium* orchids, a small number of studies have reported on the mitogenome's structure, but comprehensive, comparative mitogenomic analyses across multiple species in the genus are still lacking. Some studies noted significant structural differences in their mitogenomes, with evidence for rearrangements and the presence of conserved syntenic regions. For example, *D. loddigesii* was shown to have a complex mitogenome with several rearranged regions and a unique gene content[14]. Similarly, *D. hancockii* has been found to exhibit a high level of structural variation in the mitogenome, with large inversions and potential evidence of IGT[15].

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In this study, we present a detailed comparative analysis of the mitochondrial genomes from four *Dendrobium* species (*D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum*). For the mitogenome assembly and annotation of *D. huoshanense* and *D. chrysotoxum*, a combined strategy was employed using Illumina, PacBio and Oxford Nanopore Technologies (ONT) sequencing data to ensure the accuracy of the assemblies. For *D. lasianthera* and *D. tangerinum*, high-fidelity (HiFi) sequencing data were utilized, providing high-fidelity long reads for more precise mitogenome assembly. This approach enabled comprehensive assemblies and annotations for each species, allowing us to explore the structural diversity of these mitogenomes, assess conserved and rearranged syntenic regions and evaluate the extent of IGT. By comparing these four species, we aim to uncover the evolutionary forces shaping the mitogenomic architecture in *Dendrobium*, shedding light on the role of gene flow and structural variation in their evolution.

Materials and methods

Data source

Raw sequencing data used for mitogenome assembly in this study were retrieved from the National Center for Biotechnology Information's (NCBI's) Sequence Read Archive (SRA) (www.ncbi.nlm.nih.gov/sra). For *D. huoshanense* and *D. chrysotoxum,* raw reads were obtained from previously published studies (BioProject IDs PRJNA1129804 and PRJNA1194330, respectively), and the mitogenomes were reassembled using both short and long reads. For *D. lasianthera* and *D. tangerinum*, mitogenomes were newly assembled using HiFi long-read data. A full list of all *Dendrobium* mitogenomes included in this study, including genome features, assembly methods, platform and BioProject IDs, is provided in Supplementary Table S1.

Mitogenome assembly and annotation

For the *D. huoshanense* and *D. chrysotoxum* mitochondrial analyses, clean Illumina paired-end (PE) reads obtained via whole-genome sequencing were randomly selected using SeqKit 0.13.1[16] to create datasets for mitogenome assembly. A combined strategy was employed to ensure the accuracy of the mitogenome assembly. The Illumina reads were assembled *de novo* using SPAdes 3.15.2[17], with k-mer values set at five different values (51, 71, 91, 101 and 121) to obtain assembled scaffolds. The coding sequences (CDS) from the *D. brymerianum* mitogenome (LC704589.1-LC704614.1) were used as query sequences for a BLAST2.11.0+[18] search against the draft scaffolds. The non-mitogenomic scaffolds were excluded by visualizing the results in Bandage 0.8.1[19]. Mitogenomic sequences were selected by filtering out fragments with abnormal read depths when compared with the expected mitochondrial depths (10 times or more lower than nuclear genome sequences and 100 times or more lower than plastome sequences). These mitogenomic sequences were used to select PacBio or ONT reads by conducting a BLAST search (80% identity) with the corrected PacBio or ONT reads. These sequences were self-corrected using NextDenovo 2.3.1 (https://github.com/Nextomics/NextDenovo), followed by *de novo* assembly using Flye 2.8.3[20]. The assembled mitogenome graph was visualized in Bandage 0.8.1. The final mitogenome annotations were performed using Geseq (https://chlorobox.mpimp-golm.mpg.de/geseq.html) with *D. brymerianum* (LC704589.1–LC704614.1) as a reference genome for the mitogenome. Manual verification and corrections were performed for all annotations to ensure accuracy. The genome maps were generated using OGDRAW 1.3.1 (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html). For the *D. lasianthera* and *D. tangerinum*, HiFi sequencing data were used for mitogenome assembly. The HiFi sequencing data were processed using the assembly tool PMAT[21] to generate accurate and high-quality mitogenome assemblies. This approach leveraged the high accuracy and long-read capabilities of HiFi sequencing, providing better resolution and fewer assembly gaps compared with short-read sequencing data. The mitogenome assembly was performed by initially correcting the HiFi reads using a quality correction tool and then assembling them using Flye 2.8.3[20]. The PMAT tool was employed to ensure the correct identification and assembly of the mitogenomes from the HiFi data. The annotation process followed the same approach as described for the *Dendrobium* species, using Geseq for annotation, with appropriate reference genomes for the mitogenome. All annotations were verified manually to ensure the accuracy of the final genomic features.

Repeats detection

Dispersed repeats were identified using REPuter (https://bibiserv.cebitec.uni-bielefeld.de/reputer) with the following parameters: Hamming distance = 3, minimal repeat size = 30, a sequence identity threshold of 90% or greater, a maximum of 5,000 computed repeats and an e-value cutoff of $1e^{-5}$[22]. Simple sequence repeats (SSRs) were detected using the MISA tool[23], with motif sizes ranging from one to six nucleotides. The repeat unit thresholds were set to eight, five, four, three, three and three for mono-, di-, tri-, tetra-, penta- and hexa-nucleotide SSRs, respectively. In addition, we set the maximum sequence length between two SSRs for compound SSR detection to 100 base pairs, ensuring accurate identification of compound SSRs. REPuter was also used with default settings to identify long repeats within the plastid genome.

Examination of synteny and transfer of genomic fragments

To identify transferred fragments between the plastome and mitogenome, BLAST 2.11.0+ was used to search for homologous fragments with an e-value cutoff of $1e^{-5}$ and a minimum identity threshold of 80%, as described previously[24]. Interspecies homologous regions were analyzed to assess mitogenomic synteny among the four orchid species, excluding fragments shorter than 100 bp. The results were visualized using the Circos package within TBtools[25].

Phylogenetic analysis

Phylogenetic analysis was performed on the mitogenome sequences of the four *Dendrobium* species, along with other published orchid species and 11 monocot species. In addition to the newly sequenced species, the mitogenomic data of other relevant species were retrieved from the NCBI. These included species representing various clades in the angiosperm tree of life, such as *D. nobile* (NC_082591), *D. officinale* (OR413932.1), *D. henanense* (LC744563), *D. gratiosissimum* (LC660223), *D. wilsonii* (LC744539), *D. moniliforme* (LC660342), *D. lohohense* (LC660322), *D. aphyllum* (LC660274), *D. loddigesii* (PP829191), *D. primulinum* (LC660005), *Cymbidium ensifolium* (OR754281), *C. macrorhizon* (OQ029563.1), *Spirodela polyrhiza* (NC_017840.1), *Butomus umbellatus* (NC_021399.1), *Zea mays* NB (AY506529.1), *Allium cepa* (NC_030100.1), *Crocus sativus* (OL804177.1), *Zea perennis* (NC_008331.1), *Phoenix dactylifera* (NC_016740.1), *Oryza sativa japonica* (BA000029.3), *O. minuta* (NC_029816.1) and *Arabidopsis thaliana* (JF729202.1) (used as the outgroup). To prepare the data for phylogenetic analysis, all shared coding sequences (CDS) were extracted and aligned using MEGA 7[26]. For the mitogenomes, 33 CDSs were used in the alignments. The aligned sequences were then concatenated using Geneious Prime (www.geneious.com). To identify the optimal regions for alignment, Gblocks v.0.91b was used with the default parameters[27], ensuring the selection of the most informative regions. For phylogenetic inference, a maximum likelihood (ML) approach was used in IQ-Tree 2.1[28], employing the best-fitting evolutionary models as determined by the Bayesian information criterion (BIC) scores. The model selected for the

mitogenomes was chosen 'GTR + F + G4'. To assess the robustness of the phylogenetic tree, 1,000 bootstrap (BS) replicates were performed. The resulting phylogenetic tree was visualized in iTOL6 (https://itol.embl.de/) in Newick format.

Results

Gene content variation and structural diversity in the mitogenome of orchid species

The mitogenome sizes of the four assembled orchid species ranged from 485,249 bp (*D. lasianthera*) to 773,276 bp (*D. huoshanense*), with GC contents between 43.8% and 44.7%. The coding regions accounted for only 4.6%–7.5% of the total genome length, indicating a high proportion of noncoding sequences. A total of 60–65 genes were identified across these genomes, including 36–39 protein-coding genes (PCGs), 21–25 tRNA genes and 3 rRNA genes (Table 1, Supplementary Table S2). Among them, nine protein-coding genes contained introns including *ccmFc, cox2, nad1, nad2, nad4, nad5, nad7, rps3* and *rps10*.

Comparative analysis with 10 previously published *Dendrobium* mitogenomes revealed that the newly assembled genomes fall within the expected size and gene content range for the genus. However, gene content variations were observed; for example, *D. huoshanense* contained only 36 PCGs in this study, whereas a previous report annotated 38 PCGs. These differences likely reflect both methodological variation in annotation and genuine biological processes, such as gene loss, pseudogenization or IGT (Supplementary Fig. S1).

Gene content comparisons across nine *Dendrobium* species and four additional orchid species (*V. planifolia*, *C. ensifolium*, *C. lancifolium* and *C. macrorhizon*) revealed both gene duplications and losses (Fig. 1b). Several genes, including *atp1*, *atp6*, *ccmFN*, *cox1*, *cox3*, *mttB*, *nad1*, *nad2*, *nad3*, *nad9*, *rpl16*, *rps12*, *rps14* and *sdh4* were found in duplicated forms. In some cases, these copies differed in length; for instance, *atp6* was 60 bp longer than *atp6-b*, and *rpl16* was 294 bp longer than *rpl16-b* in the *D. tangerinum* mitogenome. However, most duplicated genes were identical in sequence. The *rpl10* and *sdh3* genes were absent in all *Dendrobium* species, as well as four additional orchid species (*V. planifolia*, *C. ensifolium*, *C. lancifolium* and *C. macrorhizon*). The *rps7* gene was lost from the mitogenomes of *D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum,* whereas *rps12* and *rps14* were lost from *V. planifolia*. The results indicated that both gene losses, duplications and variations both existed in these orchid species. Gene loss events were observed in the *Dendrobium* mitogenomes, consistent with patterms previously reported in other angiosperms, especially orchids. Some gene losses, such as *rpl10* and *sdh3*, were shared across all analyzed orchid species, indicating conserved patterns of gene loss. These findings highlight the extensive gene content variation in orchid mitogenomes, driven by gene loss, duplication and structural divergence, and underscore the dynamic nature of the mitochondrial genome's evolution in Orchidaceae.

Table 1. Features of four *Dendrobium* mitogenomes.

Species	*D. lasianthera*	*D. tangerinum*	*D. huoshanense*	*D. chrysotoxum*
Size (bp)	485,249	535,423	773,326	568,767
Total GC content	44.7%	43.8%	44.3%	44.0%
Coding GC	44.1%	44.3%	43.3%	43.0%
Non-coding GC)	43.2%	43.0%	43.6%	42.8%
Genes	65	65	60	61
tRNA	23	25	21	22
rRNA	3	3	3	3
Protein-coding genes	39	37	36	36

Repetitive sequences reveal structural diversity in *Dendrobium* mitochondrial DNA

The mitochondrial DNA (mtDNA) of flowering plants, including members of the *Dendrobium* genus, is known for its structural complexity, often driven by the abundance of repetitive elements. In this study, we identified a total of 1,478 SSRs across the mitogenomes of four *Dendrobium* species. The distribution of SSRs across the species is as follows: *D. lasianthera* had 284 SSRs, *D. huoshanense* had 231, and *D. tangerinum* and *D. chrysotoxum* each had 333 SSRs. Among these, tetranucleotide motifs were the most common SSR type, representing 12.68% of total SSRs in *D. lasianthera*, 10.81% in *D. tangerinum*, 21.65% in *D. huoshanense* and 14.41% in *D. chrysotoxum*. Hexanucleotide repeats were rare or absent, with no instances observed in *D. lasianthera*, *D. tangerinum* or *D. huoshanense*. Homopolymer SSRs with A/T motifs were also abundant across all species (Fig. 2). In addition to SSRs, 167 tandem repeats were detected, distributed as follows: 30 in *D. lasianthera*, 22 in *D. tangerinum*, 82 in *D. huoshanense* and 33 in *D. chrysotoxum*. A total of 5,069 dispersed repeats were also identified, comprising 56.80% forward repeats and 42.90% palindromic repeats across the four mitogenomes. Reverse repeats were rare: *D. tangerinum* harbored a single 33-bp reverse repeat (0.36% of its 275 total dispersed repeats) and *D. chrysotoxum* contained three long reverse repeats (30, 33 and 33 bp), representing 0.51% of its 584 dispersed repeats (Supplementary Tables S3–S6). Notably, certain mitogenome chromosomes within these species lacked any dispersed repeats, indicating distinct structural configurations and potentially altered recombinational dynamics. Overall, the abundance, distribution and diversity of repeat sequences reflect substantial structural variation in *Dendrobium* mitogenomes, underscoring their dynamic and complex evolutionary history.

Species-specific patterns of repeat lengths in *Dendrobium* mitogenomes

The distribution of repeat lengths in the mitogenomes of *D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum* revealed a clear predominance of short repeats (30–100 bp) across all species. *D. huoshanense* exhibited the highest count in this category, with 3,485 repeats, reflecting an exceptionally high density of short repeats in its mitogenome. *D. chrysotoxum* also showed a notable peak in this range, with 416 repeats, though far fewer than *D. huoshanense*. In contrast, *D. lasianthera* (292 repeats) and *D. tangerinum* (243 repeats) contained comparatively fewer short repeats.

Longer repeat elements were progressively rarer across all four mitogenomes. Within the 101–300 bp range, *D. huoshanense* again had the highest count (291 repeats), followed by *D. chrysotoxum* (123), while *D. tangerinum* and *D. lasianthera* each had fewer than 20. A similar trend was observed in the 301–500 bp category, with *D. huoshanense* (40) and *D. chrysotoxum* (32) again leading. Repeats longer than 500 bp were rare overall; *D. huoshanense* was the only species with notable numbers in these size classes, including 34 repeats between 501 and 1,000 bp, and 29 repeats over 1,000 bp. Other species exhibited only single-digit counts in these longer repeat categories (Fig. 3).

Overall, the mitogenomes of these *Dendrobium* species are characterized by a strong dominance of short repeat sequences (30–100 bp), with *D. huoshanense* exhibiting the highest repeat density across all size ranges. This pattern highlights both the prevalence of small repeats in orchids' mitochondrial genomes and the species-specific accumulation of larger repeats, particularly in *D. huoshanense*, which appears to underline its unique genomic structure and evolutionary dynamics.

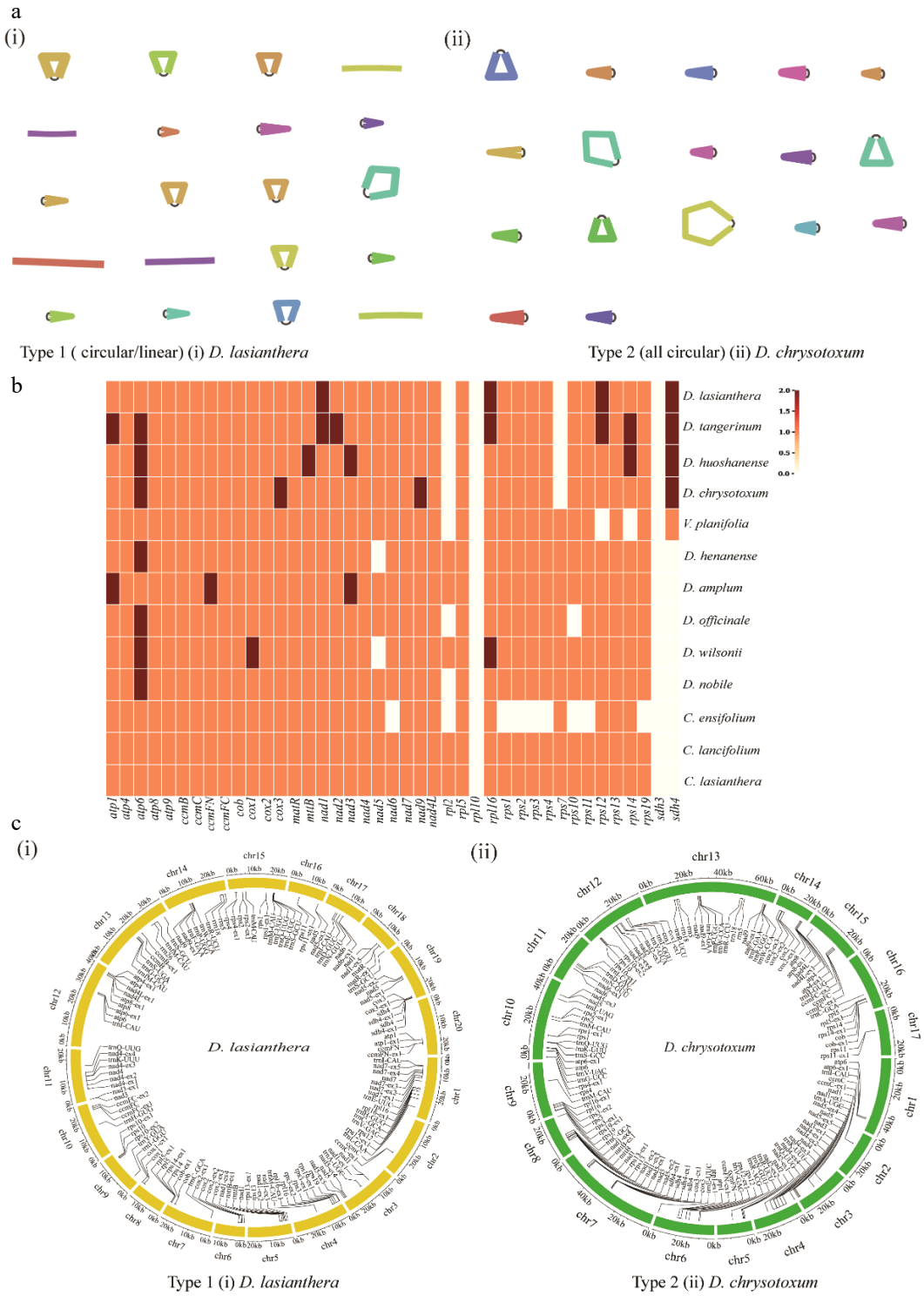

Fig. 1 (a) Structure of the mitochondrial genome of (i) (Type 1) *D. lasianthera* and (ii) (Type 2) *D. chrysotoxum*. (b) Comparison of the mitochondrial protein-coding gene contents in nine *Dendrobium* species and four additional orchid species. Colors indicate single-copy (orange), double-copy (red) and lost genes (empty). (c) Mitochondrial genome maps of (i) (Type 1) *D. lasianthera* (ii) (Type 2) *D. chrysotoxum*.

Synteny reveals conserved and species-specific evolution in *Dendrobium* mitogenome

In this study, we analyzed the mitogenome synteny among four *Dendrobium* species: *D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum*. The synteny analysis was conducted by comparing genomic regions across species to identify conserved segments that reflect evolutionary relationships. Multiple syntenic links were identified across the species (Fig. 4), suggesting genomic conservation among them.

Our analysis identified several syntenic links between the chromosomes of *D. lasianthera* and *D. tangerinum*. Notably, a significant syntenic region was observed between the chromosomes of *D. lasianthera* (Positions 295,226 to 310,516) and *D. tangerinum* (Positions 245,589 to 230,297), suggesting a shared evolutionary origin

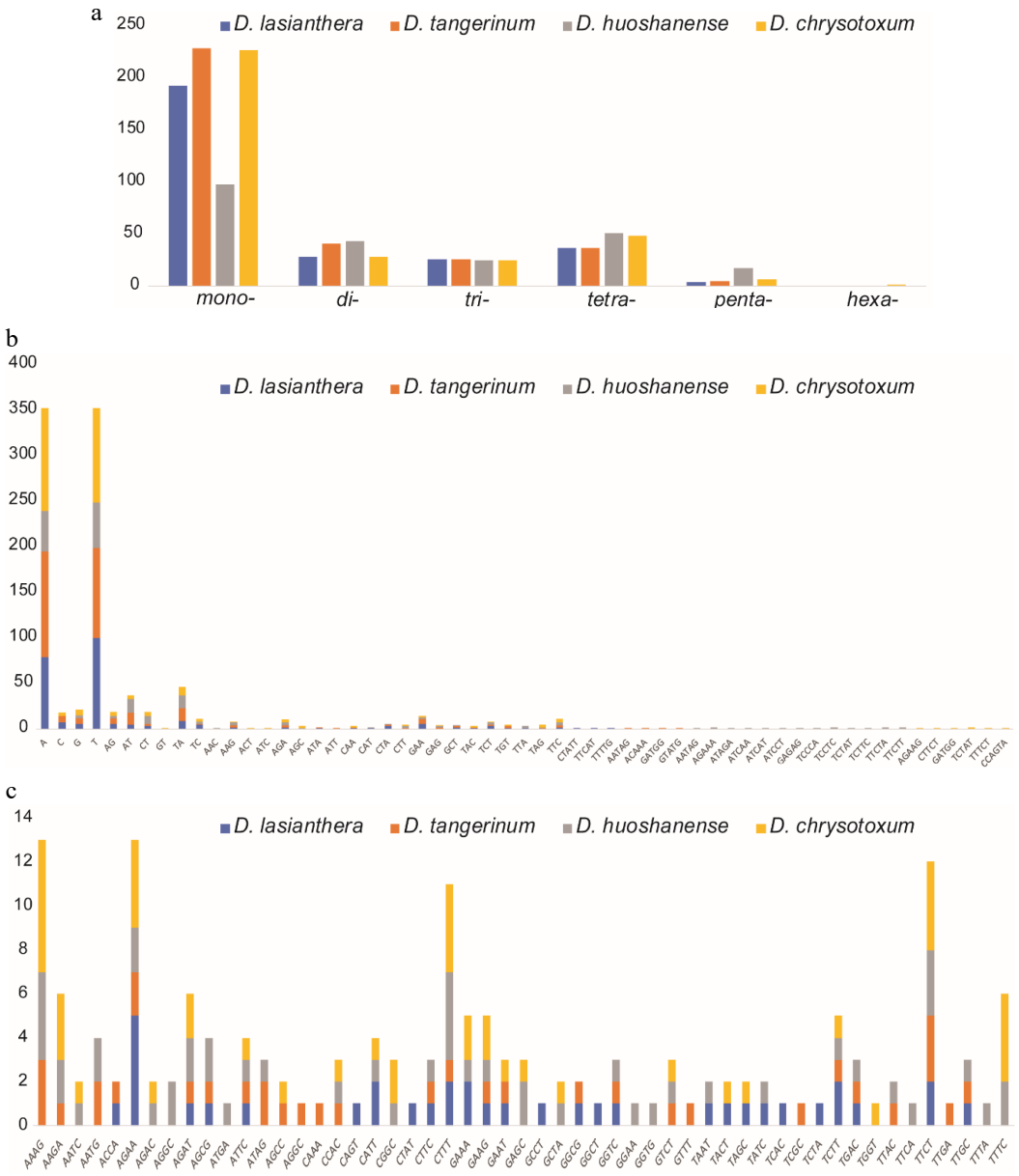

Fig. 2 SSRs in the mitogenomes of four *Dendrobium* species. (a) Count of SSRs from six different motif types. (b) Count of different motifs acorss four *Dendrobium* species. (c) Count of tetra-nucleotide SSRs in four *Dendrobium* species.

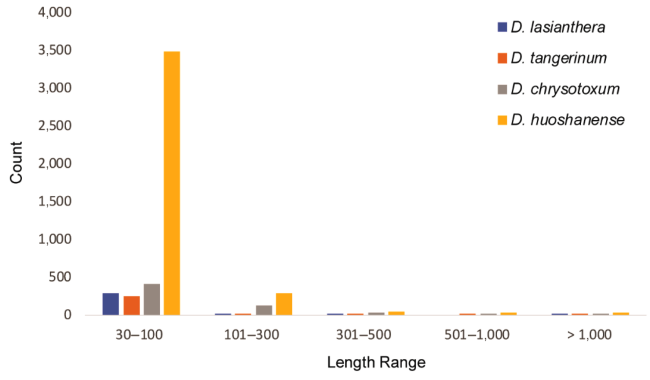

Fig. 3 Length of dispersed repeats of the mitochondrial genome in four *Dendrobium* species.

for these regions. A second syntenic link was detected between *D. lasianthera* (Positions 402,934 to 415,703) and *D. tangerinum* (Positions 112,683 to 125,424), further supporting the genetic relatedness between these two species. Another conserved chromosomal segment was found between *D. lasianthera* (Positions 141,131 to 152,644) and *D. tangerinum* (Positions 473,763 to 462,263), which indicates additional shared genomic features. Furthermore, a fourth syntenic link was detected between *D. lasianthera* (Positions 199,325 to 209,646) and *D. tangerinum* (Positions 3,876 to 14,189), underscoring the chromosomal homology between these species.

Although syntenic regions were identified in other species pairs, such as *D. lasianthera* and *D. huoshanense*, as well as *D. tangerinum* and *D. chrysotoxum*, the most prominent syntenic relationships were observed between *D. lasianthera* and *D. tangerinum*. These findings suggest a closer evolutionary relationship between these two species relative to the other species in the study.

Overall, despite the fragmented nature of some assemblies, the results highlight conserved chromosomal regions, reflecting structural conservation and syntenic similarity across the species, and

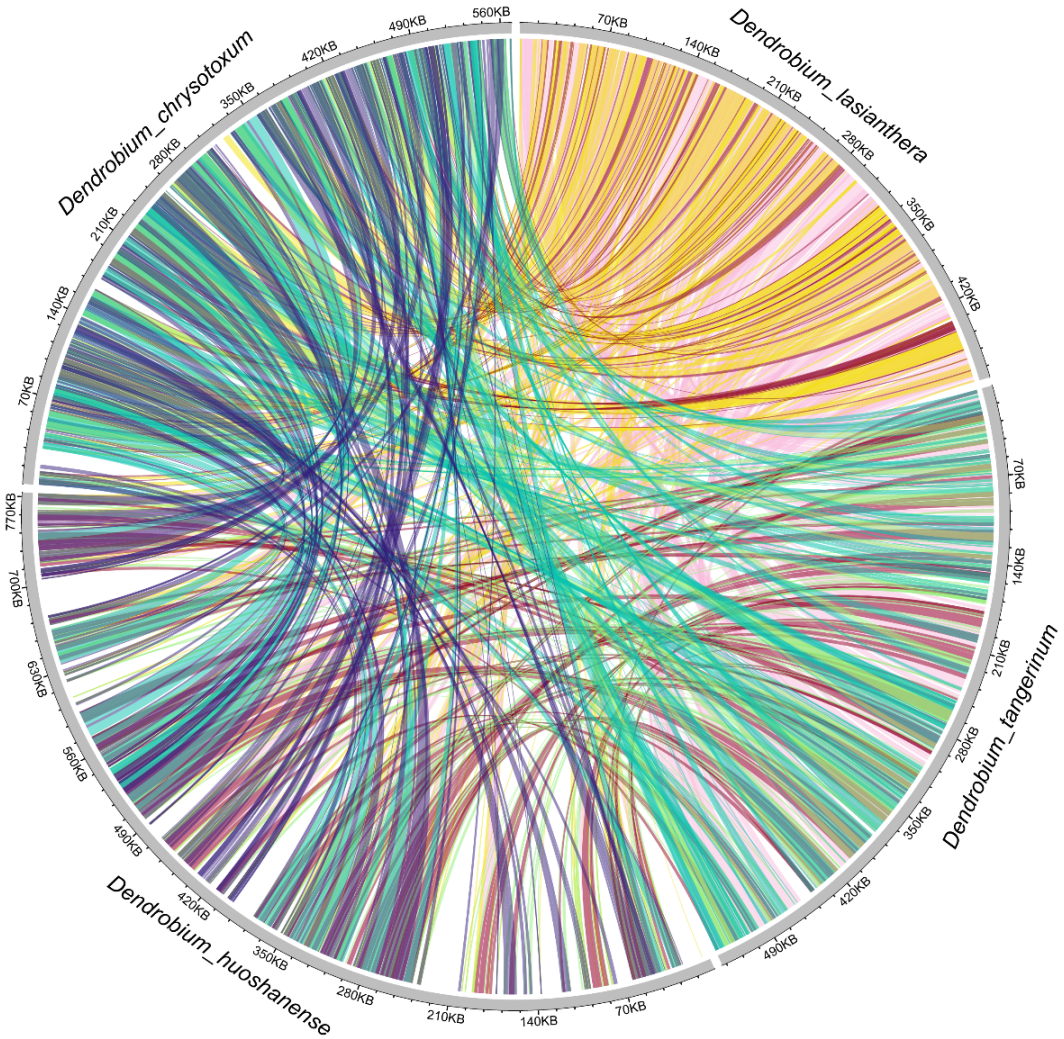

Fig. 4 Synteny among the mitochondrial genomes of four *Dendrobium* species. Interspecific mitogenomic synteny is indicated by homologous regions. The scale is shown on the outer arcs, with intervals of 70 kb.

contribute to a deeper understanding of mitogenomic evolution within the *Dendrobium* genus.

Intracellular transfers from plastids to the mitochondria in *Dendrobium*

To investigate homology between the mitogenomes and plastomes of four *Dendrobium* species (*D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum*), we conducted a comparative analysis using visualization tools. The results are presented through graphical representations of homologous regions across the mitogenomes and plastomes of these species. The analysis revealed several conserved regions between the mitogenomes and plastomes across all four *Dendrobium* species (Fig. 5). These conserved regions included key genes commonly found in plant organelle genomes, such as those involved in energy metabolism, protein synthesis and cellular respiration. The overlap of these homologous sequences highlights the functional importance of these regions in both organelles.

Key conserved transfer RNA (tRNA) genes, such as *trnN-GUU*, *trnR-ACG* and *trnI-CAU*, were identified in all four mitogenomes, showing 100% identity to their plastid copies. This strongly suggests ancestral (IGT) events that likely occurred early in the evolutionary history of *Dendrobium*. These tRNAs are typically found in the intergenic regions of the mitogenomes, suggesting their retention for functional utility in mitochondrial translation. Interestingly, *rbcL* and *psbA* fragments, which are involved in photosynthesis, were rare and found only in *D. lasianthera*, further suggesting species-specific transfer events. These results highlight the dynamic nature of organelle-to-organelle gene transfer in *Dendrobium*. Despite the overall conservation of certain gene regions, there were noticeable differences in the homologous sequences between the mitogenomes and plastomes of the *Dendrobium* species. For example, *D. chrysotoxum* exhibited more significant divergence in certain regions compared with the other *Dendrobium* species (*D. lasianthera*, *D. tangerinum* and *D. huoshanense*).

The proportion of plastid-derived sequences in the mitogenomes varied significantly across the four *Dendrobium* species. *D. huoshanense* exhibited the highest plastid-like content (36.7% of its mitogenome), followed by *D. chrysotoxum* (35.2%) and *D. lasianthera* (30.9%), while *D. tangerinum* retained the lowest proportion (22.7%). This gradient suggests species-specific dynamics in the integration of plastome-derived sequences.

Mitochondrial phylogeny highlights close and distant relationships in *Dendrobium*

A phylogenetic analysis was performed using mitochondrial CDSs from four *Dendrobium* species, along with 13 additional orchid species and 11 monocot species, to investigate their evolutionary relationships. The resulting phylogenetic tree (Fig. 6) reveals distinct clustering patterns within the *Dendrobium* species and other related taxa.

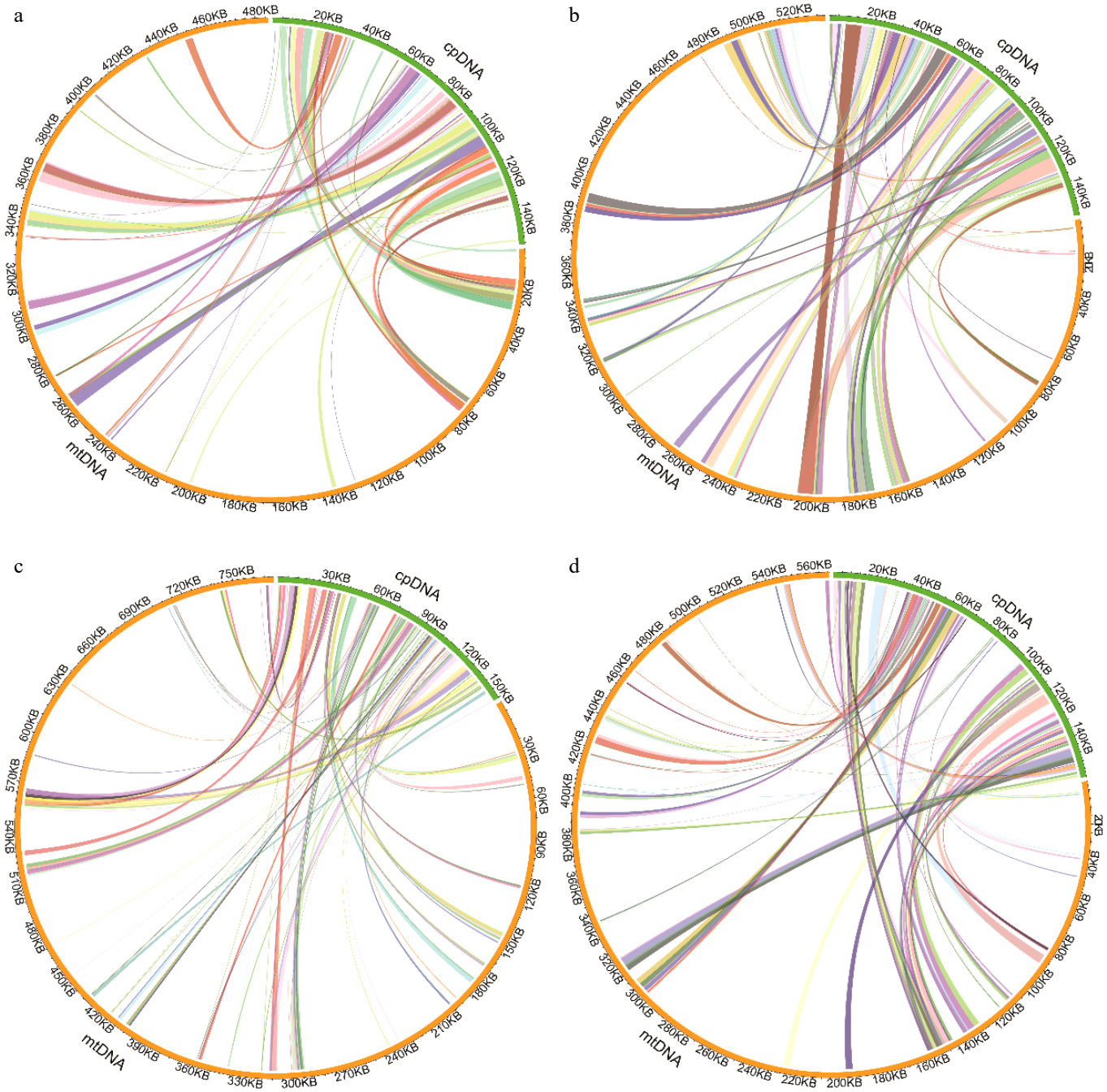

Fig. 5 Intracellular gene transfer in (a) *D. lasianthera*, (b) *D. tangerinum*, (c) *D. huoshanense* and (d) *D. chrysotoxum*. Mitogenomes are shown in orange, plastomes are showing green, and the color arcs within the circular ring represent homologous segments between the mitogenome and plastome.

The analysis showed that the *Dendrobium* species formed a well-supported clade, with several subgroups corresponding to different species. Notably, *D. lasianthera* and *D. tangerinum* were resolved as sister species, indicating a relatively recent common ancestor and a close evolutionary relationship between these two species. Similarly, *D. chrysotoxum* and *D. huoshanense* also formed a distinct subgroup within the *Dendrobium* clade, but they were more distantly related to the first group, suggesting an earlier divergence in the evolution of *Dendrobium*.

The phylogenetic tree also included several other orchid species, such as *D. nobile*, *D. officinale* and *D. wilsonii*, which are more distantly related to the species analyzed in this study. The branching pattern indicated that the *Dendrobium* species, while closely related within their genus, exhibit evolutionary divergence from other orchid genera, as was expected on the basis of sampling. Notably, the separation between the sampled orchid species and those from other plant families, such as *Zea mays* (Poaceae) and *Typha latifolia* (Typhaceae), was evident in the phylogenetic tree, supporting the close evolutionary relationship among the sampled Orchidaceae species based on mtDNA. The tree topology was well-supported by the bootstrap values, with high support for internal nodes within the *Dendrobium* clade, indicating the robustness of the phylogenetic relationships at this level of sampling. These results demonstrate that mitochondrial CDS data can resolve phylogenetic relationships within the *Dendrobium* genus. However, expanded sampling is necessary to further evaluate how mitogenomic CDS

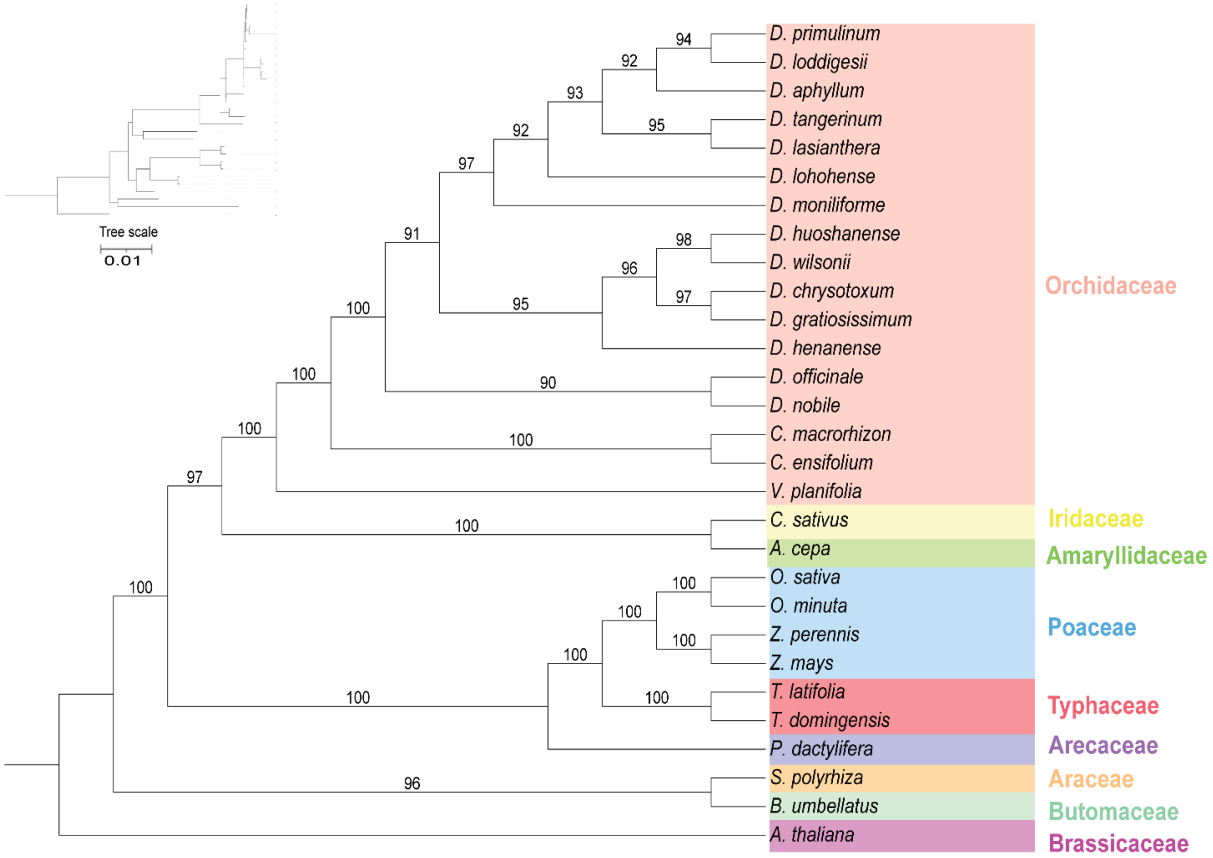

Fig. 6 Phylogenetic relationships resolved from 33 conserved DNA sequences of protein-coding genes (PCGs) in complete mitogenomes from 29 species in nine families (Orchidaceae, Iridaceae, Amaryllidaceae, Poaceae, Typhaceae, Arecaceae, Araceae, Butomaceae and Brassicaceae) in angiosperms. The number at each node is the bootstrap support value.

data perform in resolving the relationships among more closely related taxa.

Discussion

The comparative analysis of mitogenomes from four *Dendrobium* species (*D. lasianthera*, *D. tangerinum*, *D. huoshanense* and *D. chrysotoxum*) revealed substantial variation in their genome size, structural diversity, gene content variations and evidence of IGT, all of which contribute valuable insights into the evolutionary history shaping the mitochondrial genome architecture in *Dendrobium*. These species exhibited distinct configurations of circular and linear mitochondrial chromosomes, a common feature observed across plant mitogenomes. Specifically, *D. lasianthera* has 15 circular and five linear chromosomes, *D. tangerinum* 17 circular and three linear chromosomes, *D. chrysotoxum* 17 circular chromosomes and *D. huoshanense* contains 13 circular and five linear chromosomes. The presence of both circular and linear mitochondrial chromosomes in these *Dendrobium* species aligns with previous studies documenting this variability in other orchid species. For example, *D. henanense* was found to have 21 circular and three linear chromosomes, while *D. wilsonii* contains 19 circular and three linear chromosomes[29]. This structural variation is consistent with the dynamic nature of plant mitogenomes, which often exhibit both circular and linear chromosomes, with the balance between these structural arrangements varying across species[30].

The four *Dendrobium* species analyzed here exhibited notable differences in mitogenome size, ranging from 485,249 bp in *D. lasianthera* to 773,276 bp in *D. huoshanense*; GC content ranged from 43.8% to 44.7% and they harbored 36–39 PCGs and three rRNAs. These values fall within the range observed in previously sequenced *Dendrobium* species, which exhibit genome sizes from 420,538 bp to 807,551 bp and PCG counts ranging 36 to 42 (Supplementary Table S1). When expanded to include the 10 previously reported *Dendrobium* mitogenomes, it becomes clear that the mitogenomic architecture in this genus is highly variable. For instance, *D. henanense* and *D. wilsonii* were annotated with 40–41 PCGs and over 30 tRNAs, while other species such as *D. flexicaule* and *D. officinale* contained 36–37 PCGs and around 23–24 tRNAs. This variation likely reflects both biological differences (e.g., gene loss, duplication, IGT) and technical factors (e.g., differences in assembly strategies or annotation pipelines).

Despite the variation in sizes, the mitogenomes in the four species assembled in this study possess a similar gene content, including PCGs, tRNAs and rRNAs, albeit with some differences in gene content. For instance, PCGs such as *rpl2*, *rpl10*, *rps7* and *sdh3* were absent in some or all the *Dendrobium* species, suggesting that gene loss events are a common feature of orchids' mitogenomic evolution, a phenomenon frequently observed in plant mitogenomes[31,32].

A striking feature of the mitogenomes in this study is the presence of abundant SSRs, with a strong preference for tetranucleotide repeats. The repeat content varied among species, with *D. tangerinum* and *D. chrysotoxum* exhibiting higher SSR counts. This aligns with findings from other plant studies, which highlight the role of repeat sequences in shaping the mitogenome's structure and potentially facilitating structural rearrangements[11]. The presence of tandem and dispersed repeats further suggests that these elements could contribute to the genome's structural plasticity and might

play a role in adaptive evolution through mechanisms like recombination or gene transfer.

Our analysis of synteny among the four *Dendrobium* species reveals conserved genomic regions, particularly between *D. lasianthera* and *D. tangerinum*, as well as between *D. lasianthera* and *D. huoshanense*. These conserved syntenic regions indicate that these species share a more recent common evolutionary history[14]. Inversely the *Dendrobium* species pairs that were more distantly related had fewer syntenic regions. These observed patterns in synteny supports the hypothesis that mitogenomes evolve through complex mechanisms involving both conserved and rearranged regions, a pattern observed in other orchid species[15].

Our analyses of IGT between the mitogenomes and plastomes of *Dendrobium* species is important for understanding differences in deposition and retention, as well as understanding how these patterns relate to genomic architecture. Our comparative analysis of homologous sequences between these organellar genomes revealed conserved regions that are critical for energy metabolism and cellular respiration. The variation in the degree of divergence between the plastomes and mitogenomes, particularly in *D. chrysotoxum*, suggests that different evolutionary trajectories and selective pressures influenced the functional adaptations of these organelles[12,13].

Phylogenetic analysis based on coding DNA CDSs in mtDNA reinforces the evolutionary relationships within the *Dendrobium* genus. Specifically, *D. lasianthera* and *D. tangerinum* were resolved as sisters, with high bootstrap support, while *D. chrysotoxum* and *D. huoshanense* were resolved as more distantly related. These findings are consistent with previous studies that have found *Dendrobium* to be genetically diverse, with significant evolutionary variation among its species[7]. The phylogenetic tree constructed in this study demonstrates the utility of mitogenomic CDS data in resolving relationships at the species level. Moreover, these mitochondrial-based inferences complement plastid-based phylogenies and highlight the importance of integrating multiple organelle genomes for a comprehensive understanding of orchid evolution. We also note that broader taxon sampling could further improve the resolution and reliability of mitogenome-based phylogenetic frameworks for Orchidaceae.

Conclusions

This study provides a comprehensive examination of the mitogenomes of four *Dendrobium* species, revealing significant structural diversity, gene content variations and differences in IGT. We observed notable differences in genome size and composition, with certain species exhibiting conserved syntenic regions, while others showed substantial rearrangements, reflecting the dynamic nature of the mitogenome's structural evolution. The presence of SSRs and dispersed repeats further highlights the genomic plasticity that may facilitate adaptive evolution. The synteny analysis indicated shared evolutionary histories between some species, such as *D. lasianthera* and *D. tangerinum*, while others showed greater divergence. Phylogenetic analysis based on mitochondrial coding sequences reinforced these findings, showing close relationships between certain species while revealing greater genetic distance in others. Additionally, evidence of IGT between the plastome and mitogenome points to IGT as a contributing factor in mitochondrial evolution. Overall, this study improves our understanding of mitogenomic diversity in *Dendrobium*, offering valuable insights into the molecular mechanisms driving the evolution of orchid mitogenomes and providing a foundation for future research on orchids' mitochondrial biology.

Ethical statements

This study has rigorously adhered to relevant institutional, national and international guidelines and regulations. Moreover, the study did not involve the use of any endangered or protected species.

Author contributions

The authors confirm their contributions to the paper as follows: writing – original draft: Soe T, Kong J; writing – review and editing, and methodology: Soe T, Kong J, Xu B; data curation, writing – review and editing: Nie L, Kong J; writing – review and editing: Tembrock LR, Nie L, Jia M; writing – review and editing, and funding acquisition: Jia M. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated and analyzed in this article are available in the Figshare repository (https://figshare.com/) with DOI: 10.6084/m9.figshare.29434223. All other data and material analyzed in the current study are included in the manuscript and the supplementary information.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. van der Bliek AM, Sedensky MM, Morgan PG. 2017. Cell biology of the mitochondrion. *Genetics* 207:843–71
2. Fan W, Liu F, Jia Q, Du H, Chen W, et al. 2022. *Fragaria* mitogenomes evolve rapidly in structure but slowly in sequence and incur frequent multinucleotide mutations mediated by microinversions. *New Phytologist* 236:745–59
3. Zimorski V, Ku C, Martin WF, Gould SB. 2014. Endosymbiotic theory for organelle origins. *Current Opinion in Microbiology* 22:38–48
4. Lai C, Wang J, Kan S, Zhang S, Li P, et al. 2022. Comparative analysis of mitochondrial genomes of *Broussonetia* spp. (Moraceae) reveals heterogeneity in structure, synteny, intercellular gene transfer, and RNA editing. *Frontiers in Plant Science* 13:1052151
5. Gandini CL, Garcia LE, Abbona CC, Sanchez-Puerta MV. 2019. The complete organelle genomes of *Physochlaina orientalis*: Insights into short sequence repeats across seed plant mitochondrial genomes. *Molecular Phylogenetics and Evolution* 137:274–84
6. Zhang D, Zhao XW, Li YY, Ke SJ, Yin WL, et al. 2022. Advances and prospects of orchid research and industrialization. *Horticulture Research* 9:uhac220

7. Li Y, Zhang B, Zhang S, Wong CE, Liang Q, et al. 2025. Pangeneric genome analyses reveal the evolution and diversity of the orchid genus *Dendrobium*. *Nature Plants* 11:421−37
8. Wang L, Liu X, Wang Y, Ming X, Qi J, Zhou Y. 2024. Comparative analysis of the mitochondrial genomes of four *Dendrobium* species (Orchidaceae) reveals heterogeneity in structure, synteny, intercellular gene transfer, and RNA editing. *Frontiers in Plant Science* 15:1429545
9. Bleidorn C. 2016. Third generation sequencing: technology and its potential impact on evolutionary biodiversity research. *Systematics and Biodiversity* 14:1−8
10. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, et al. 2023. Next-generation sequencing technology: current trends and advancements. *Biology* 12:997
11. Sun M, Zhang M, Chen X, Liu Y, Liu B, et al. 2022. Rearrangement and domestication as drivers of Rosaceae mitogenome plasticity. *BMC Biology* 20:181
12. Choi IS, Schwarz EN, Ruhlman TA, Khiyami MA, Sabir JSM, et al. 2019. Fluctuations in Fabaceae mitochondrial genome size and content are both ancient and recent. *BMC Plant Biology* 19:448
13. Kleine T, Maier UG, Leister D. 2009. DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. *Annual Review of Plant Biology* 60:115−38
14. Tong W, Yu D, Zhu X, Le Z, Chen H, et al. 2024. The whole mitochondrial genome sequence of *Dendrobium loddigesii* Rolfe, an endangered orchid species in China, reveals a complex multi-chromosome structure. *Genes* 15:834
15. Hou Z, Wang M, Jiang Y, Xue Q, Liu W, et al. 2024. Mitochondrial genome insights into the spatio-temporal distribution and genetic diversity of *Dendrobium hancockii* Rolfe (Orchidaceae). *Frontiers in Plant Science* 15:1469267
16. Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLoS One* 11:e0163962
17. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19:455−77
18. Ye J, McGinnis S, Madden TL. 2006. BLAST: improvements for better sequence analysis. *Nucleic Acids Research* 34:W6−W9
19. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of *de novo* genome assemblies. *Bioinformatics* 31:3350−52
20. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology* 37:540−46
21. Bi C, Shen F, Han F, Qu Y, Hou J, et al. 2024. PMAT: an efficient plant mitogenome assembly toolkit using low-coverage HiFi sequencing data. *Horticulture Research* 11:uhae023
22. Kurtz S, Choudhuri JV, Ohlebusch E, Schleiermacher C, Stoye J, et al. 2001. REPuter: the manifold applications of repeat analysis on a genomic scale. *Nucleic Acids Research* 29:4633−42
23. Beier S, Thiel T, Münch T, Scholz U, Mascher M. 2017. MISA-web: a web server for microsatellite prediction. *Bioinformatics* 33:2583−85
24. Hong Z, Liao X, Ye Y, Zhang N, Yang Z, et al. 2021. A complete mitochondrial genome for fragrant Chinese rosewood (*Dalbergia odorifera*, Fabaceae) with comparative analyses of genome structure and intergenomic sequence transfers. *BMC Genomics* 22:672
25. Zhang H, Meltzer P, Davis S. 2013. RCircos: an R package for Circos 2D track plots. *BMC Bioinformatics* 14:244
26. Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870−74
27. Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17:540−52
28. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32:268−74
29. Wang M, Yu W, Yang J, Hou Z, Li C, et al. 2023. Mitochondrial genome comparison and phylogenetic analysis of *Dendrobium* (Orchidaceae) based on whole mitogenomes. *BMC Plant Biology* 23:586
30. Kozik A, Rowan BA, Lavelle D, Berke L, Schranz ME, et al. 2019. The alternative reality of plant mitochondrial DNA: one ring does not rule them all. *PLoS Genetics* 15:e1008373
31. Zhang CL, Tu XD, Wang LY, Chen DQ, Zeng MY, et al. 2025. A comprehensive analysis of the complete *Dendrobium chrysotoxum* mitogenome reveals a multiple-circular structure and horizontal gene transfer from fungi. *Ornamental Plant Research* 5:e007
32. Zheng Q, Luo X, Huang Y, Ke SJ, Liu ZJ. 2024. The complete mitogenome of *Apostasia fujianica* Y.Li & S.Lan and comparative analysis of mitogenomes across Orchidaceae. *International Journal of Molecular Sciences* 25:8151

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