Comparative analysis of the plastid and mitochondrial genomes of *Artemisia giralldii* Pamp.

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*Artemisia giralldii* Pamp. is an herbaceous plant distributed only in some areas in China. To understand the evolutionary relationship between plastid and mitochondria in *A. giralldii*, we sequenced and analysed the plastome and mitogenome of *A. giralldii* on the basis of Illumina and Nanopore DNA sequencing data. The mitogenome was 194,298 bp long, and the plastome was 151,072 bp long. The mitogenome encoded 56 genes, and the overall GC content was 45.66%. Phylogenetic analysis of the two organelle genomes revealed that *A. giralldii* is located in the same branching position. We found 13 pairs of homologous sequences between the plastome and mitogenome, and only one of them might have transferred from the plastid to the mitochondria. Gene selection pressure analysis in the mitogenome showed that ccmFc, nad1, nad6, atp6, atp7 and rps12 may undergo positive selection. According to the 18 available plastome sequences, we found 17 variant sites in two hypervariable regions that can be used in completely distinguishing 18 *Artemisia* species. The most interesting discovery was that the mitogenome of *A. giralldii* was only 43,226 bp larger than the plastome. To the best of our knowledge, this study represented one of the smallest differences between all sequenced mitogenomes and plastomes from vascular plants. The above results can provide a future reference for taxonomic and molecular evolution studies of Asteraceae species.

*Artemisia* is one of the largest and most widely distributed genera in the family of Asteraceae. It is a heterogeneous genus consisting of more than 500 different species distributed mainly in Europe, Asia and North America1–2. These species are perennial, biennial and annual herbs or small shrubs2–4. Its pungent odor and bitter taste are due to terpenoids and sesquiterpene lactones5. Some *Artemisia* species are cultivated as crops, whereas others are used in preparing tea, tonic, alcoholic beverages and medicines6. Various biochemically active secondary metabolites have been identified in *Artemisia* species, including essential oils, flavonoids, terpenoids, esters and other substances7–9, which are potential bioactive compounds for developing novel herbal drugs against multiple diseases, such as cancer10, malaria11, hepatitis12, inflammation13 and fungal, bacterial14 and viral infections14. Researchers have extracted artemisinin from *Artemisia annua* and demonstrated its antimalarial effects15. Tu et al. converted artemisinin into a drug that has saved millions of lives worldwide16, thus winning the 2015 Nobel Prize in medicine. These researchers have confirmed the medicinal value of *Artemisia* species and its potential use in bio-exploration.

*Artemisia giralldii* Pamp. is one of the 186 *Artemisia* species found in China. It is an herbaceous plant distributed only in some areas of China (e.g., Henan, Hebei, Gansu, Ningxia, Shanxi and Sichuan Provinces). Studies on *A. giralldii* are few and have mainly focused on its chemical composition, geographical distribution17 and community18. The main chemicals in *Artemisia* are terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes. Two flavones and several monoterpenoids and sesquiterpene lactones have been isolated from the aerial parts of *A. giralldii*19. Two of these flavones named 4′, 6, 7-trihydroxy-3′,5′-dimethoxyflavone and 3′, 5′-dihydroxy-3′, 4′, 8-trimethoxyflavone showed antibiotic activity against *Escherichia coli*, *Sarcina lutea*, *Pseudomonas aeruginosa* and *Aspergillus flavus*. A monoterpenone, called santolinylol, which has antifungal activity, has been isolated from *A. giralldii*20. The flowering parts of *A. giralldii* are rich in essential oils. Studies have shown that these essential oils exhibit strong fungicidal activity against *Sitophilus zeamais* adults and possessed substantial contact toxicity against maize weevils21.

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Molecular breeding, genetic engineering and synthetic biology of *Artemisia* species have attracted considerable interest, which are critical to obtaining active materials efficiently. The first steps for genetic studies include sequencing and analysing the nuclear and organelle genomes.

Mitochondria and plastids originate from bacterial endosymbionts. The convergent evolution of mitochondria and plastid can be observed between distantly related species, the same strain and even within the same cell. However, although mitochondrial and plastid genomes follow similar evolutionary paths, mitochondrial genomes have evolved much further. The mitochondrial genome (mitogenome) is more complex than the plastid genome and more severe gene loss, more extensive and refined forms of post-transcriptional editing and processing, more gene isoforms and a wider range of gene fragmentation in most photosynthetic plants. However, the number of plastid genes is not larger than that of mitochondrial genes in some plants. In some non-photosynthetic plants, such as *Hypopitys monoptera* or *Rhopalocnemis phalloides*, the plastomes showed considerable gene loss and size reduction. The plastome size decreases up to 110–200 kb in autotrophic plants.

Then, we applied a hybrid strategy combining Illumina and Nanopore reads. First, we used the Illumina data alone to assemble the plastome. The parameters applied for plastome were ‘-R 15 -k 20’. Co-extension/coexistence of mitochondrial and plastid genomes was observed in various species, and in most cases, plastid DNA was overtaken by mitochondrial DNA. We can identify the interaction between the two organelles from the comparative analysis of mitochondrial and chloroplast genomes of the same species.

The animal mitogenome is normally a circular, compact molecule about 17 kb long with little variation in size. It contains about 13 protein-coding genes (PCGs), two ribosomal RNAs (rRNA) genes and 22 or 23 transfer RNA (tRNA) genes among bilaterians, with a few exceptions. Although much larger mitochondrial genomes have occasionally been found, they are usually the product of duplicating portions of the mtDNA rather than variation in gene content. Unlike the relatively simple animal mitochondrial genomes, non-parasitic flowering plant mitochondrial genomes were large and complex. They exhibit a wide range of variations in size, sequence alignment and repeat content, but the coding sequences are highly conserved (typically 24 core genes and 17 variable genes). Usually, the mitogenome was represented as a monomeric circle with no mention of other forms as circular mapping is a convenient indicator of genome content and sequencing completion. Thus, the circular map appears in published plant mitochondrial genomes. However, plant mitochondrial DNAs appear as linear and multi-branched molecules under electrophoresis and microscopy. At the same time, some studies have also proposed that plant mitochondria are non-circular. They are a collection of multiple forms, including circular, linear and branching molecules. Some of these molecules might represent the intermediate molecule of replication or recombination. Multiple forms can also be called isomers of the genome. The cause of isomers may be the frequent recombination of some repetitive sequences in the plant mitochondrial genome promoting rearrangement of the genome, which is also indirectly indicated by the near-complete disruption of gene order among closely related species. Cytoplasmic male sterility (CMS) is the most evident and widespread phenotype associated with plant mitogenomic rearrangements. CMS has long been of interest to plant breeders because the male-sterile phenotype contributes to hybrid seed production. Mining whole mitogenome sequences can complement the experimental approaches. In particular, they can reveal the origin, expression and evolution of CMS genes and the effect of CMS on mitogenome evolution.

Seven thousand three hundred sixty-three complete plastomes and 423 plant mitogenomes have been recorded in the GenBank Organelle Genome database (https://www.ncbi.nlm.nih.gov/genome/browse) (last updated: December 20, 2021). The structural complexity of mitochondria results in significantly more difficulty in their genome assembly. Only a few mitochondria mitogenomes have been reported. Until now, no mitogenome in the *Artemisia* genus has been reported. This deficit has limited our understanding of the evolution and functioning of the mitochondria in this genus. Here, we assembled and annotated the plastome and mitogenome of *A. giralldii* for the first time. We analysed the gene content, repeat sequence and selection pressure of the *A. giralldii* mitogenome. In addition to these, we attempted to understand the evolving relationship between the plastomes and mitogenomes of Asteraceae species by constructing phylogenetic trees of 10 Asteraceae species. Lastly, we analysed the homologous sequence between the two organelle genomes. The results obtained from this study provide the first account of the mitogenome structure and shed light on the interaction between the mitogenome and plastome.

**Materials and methods**

**Plant materials and DNA extraction and sequencing.** We collected fresh *A. giralldii* Pamp. Leaves from the Institute of Medicinal Plant Development (IMPLAD), Beijing, China. Then, the total genomic DNA (accession number: implad201910017) was extracted using a DNA extraction Kit (Tiangen Biotech, Beijing, China) and stored in a refrigerator at −80 °C. A DNA sequence library was constructed with 1 ug of DNA by using a NEBNext library building kit and sequenced with a 2500 platform (Illumina, San Diego, CA, USA). Clean data were obtained by removing low-quality sequences with Trimmomatic software under the following conditions: sequences with more than 50% bases with quality values (Q) of <19 and more than 5% N bases. The plant sample used for Illumina short-read sequencing was subsequently used for Oxford Nanopore sequencing. Raw reads obtained by Nanopore sequencing were filtered to remove reads with Q of <7. Genomic DNA was prepared using the CTAB method and purified with a QIAGEN genomic kit (Cat# 13343, QIAGEN) according to the standard operating procedure provided by the manufacturer. About 700 ng of DNA was used in library construction and then sequenced on a Nanopore PromethION sequencer instrument (Oxford Nanopore Technologies, UK) at the Genome Center of Grandomics (Wuhan, China).

**Genome assembly and annotation.** GetOrganelle was used in assembling the organelle genomes. We first used the Illumina data alone to assemble the plastome. The parameters applied for plastome were ‘-R 15 -k 21, 45, 65, 85, 105 -F emplant.pt’. Then, we applied a hybrid strategy combining Illumina and Nanopore reads to assemble the mitogenome. GetOrganelle was used in extracting mitochondrial genome reads from Illumina.
whole-genome sequence (WGS) data. We then assembled the extracted reads into a unitig graph. All the 'edges' of the unitig graph had the same coverage depth, suggesting the absence of plastid and nuclear sequences, which tend to show significantly higher or lower coverage. The unitig graph contained multiple double-bifurcation structures (>= <). DBSs resulting from the presence of repeat sequences in the genome. To resolve the sequence path around these DBSs, we constructed all possible sequences around the Nanopore reads with minimap2 tool.48. For each DBS, we selected the sequence path with the largest number of Nanopore reads mapped as the dominant sequence path. Finally, we identified a cyclic path on the unitig graph covering all the 'edges'. This path corresponded to a circular DNA sequence, which was considered the mitogenome.

The plastome was annotated using CPGAVAS29, and the reference genome was *Chrysanthemum indicum* (NC_020320.1)30. The diagrams of cis-splicing and trans-splicing genes in the plastome were created using CPGview (http://www.herbalgenomics.org/cpgview). The mitogenome was annotated using MGAVAS (http://www.1kmpg.cn/mgavas) and GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq.html)31, and its reference genome was *C. indicum* (MH716014.1)32. We annotated the mitogenome using MGAVAS (http://www.1kmpg.cn/mgavas/) and tRNAscan-SE39 with default settings to confirm the annotations. We used Apollo34 to manually correct the annotation problems and OrganellarGenomeDRAW (OGDRAW) (v1.3.1)35 to draw a genome map. Then, we submitted the organelle genome sequences and annotations to GenBank by BankIt (https://www.ncbi.nlm.nih.gov/WebSub/) and obtained accession numbers OK128342 for the plastome and NC_064134.1 for the mitogenome.

Homology sequence analysis between plastid and mitochondrion. Sequence similarity comparison between the plastome (OK128342) and mitogenome (NC_064134.1) was carried out for the identification of homologous sequences between two organelles. BLASTN was used, and the e-value cutoff was 1e−536. The final results were visualised using the Circos package implemented in TBtools37,38.

Repeat elements analysis. The microsatellite sequence repeats were identified by using Misa (https://weblast.ipk-gatersleben.de/misa/) with the parameters ‘1-10 2-5 3-4 4-3 5-3 6-3’39. The tandem repeats were identified using TRF with the following parameters: ‘2 7 80 10 50 50 -f -d -m’40. The dispersed repeats were identified using REPiter web server (https://bibiserv.zceb.tuebingen.mpg.de/reptiter/) with the following parameters: hamming distance, 3; maximum computed repeats, 5000; and minimal repeat size, 30 and filtered at an e-value of 1e−441. Visualisation was conducted according to the procedure for homologous sequence analysis.

Phylogenetic inference analysis. The plastome and mitogenomes of *A. giralldii* combined with 11 Asteraceae species were used in phylogenetic analysis. Two *Salomon* genus species were selected as outgroup taxa. The common genes of 12 species were extracted using Phylosite (v1.1.16)42. From the plastome, we extracted the coding sequences from 67 common genes (atpA, atpB, atpE, atpF, atpH, ccsA, ccmA, matK, ndhA, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhI, ndhJ, ndkA, petA, petB, petD, petG, petL, petN, psaA, psaB, psaC, psaI, psaJ, psbA, psbB, psbD, psbF, psbH, psbI, psbJ, psbK, psbM, psbN, psbT, rbcL, rpl2, rpl14, rpl16, rpl20, rpl22, rpl32, rpl33, rpl36, rpoA, rpoB, rpoC1, rpoC2, rps2, rps3, rps4, rps7, rps8, rps11, rps12, rps15, rps16, rps18, rps19, rps20, rps22, rps23, rps24, rps26, rps27, rps3, rps4, rps5 and ycf4) from 10 Asteraceae species and two outgroup taxa from the plastome. From the mitogenome, we extracted 29 orthologous mitochondrial genes (atp1, atp4, atp6, atp8, cox1, cox2, cox3, ccmB, ccmC, ccmF, ccmF1, cytB, matR, mttB, nad1, nad2, nad3, nad4, nad4L, nad5, nad6, nad7, nad9, rpl10, rps3, rps4, rps12 and rps13) from the same set of species for analysis. Then, we aligned the coding sequences with MAFFT (v7)45 and concatenated them with Phylosite (v1.1.16). We used Gblocks with default parameters to optimise the alignment of the concatenated sequences46. The phylogenetic tree was built using the maximum-likelihood method implemented in IQ-TREE (v2)47 and visualised using iTOL (v5; https://itol.embl.de/)48. Bootstrap analysis was performed using UFBoot with 1000 replicates49. The best model was selected using jModelTest (v2.1.0)50 according to the Bayesian information criterion. TVM + G was found to be the best model for plastome and mitogenome analyses. We performed Bayesian inference (BI) analysis using MrBayes (v3.2.7)51. The BI tree was visualised using iTOL (v5)52.

Selective pressure analysis of *A. giralldii* mitogenome. We used EasyCodeML (v1.4) software53 to conduct the selective pressure analysis of 28 protein-coding genes in the mitogenome. The running model was ‘Preset (Nested Models)’. The site model in EasyCodeML can be used in identifying positively selected sites in a multiple-sequence alignment54. The required inputs for analysing selection are aligned sequences in PAML format and a tree file in Newick format. Firstly, we aligned each gene from 10 species with MAFFT (v7)55 and converted the alignment into PAML format by using the ‘Seqformat Converter’ tool in EasyCodeML (v1.4). Then, we used IQ-TREE (v2)56 to generate a tree file in Newick format. Finally, we ran the CodeML with the following parameters: nt = 0 and icode = 0. On the basis of the InL and np values of the null model (M0, M1a, M7 and M8a) and alternative model (M3, M2a and M8), the likelihood ratio test (LRT) p-value of each PCG was calculated. Then, the p-values were adjusted using the Benjamini–Hochberg correction method57. Genes with adjusted p-values of <0.05 were considered positively selected.

Molecular marker development. To discover universal primers that can be used in distinguishing the *Artemisia* species, we downloaded the 17 plastome sequences of *Artemisia* species from GenBank. They were analysed using ecoPrimers58 with the following parameters: ‘-l 300 -L 600 -e 0 -3 2 -t species -U -f -O 25’. Here, ‘-l 300’ specified the minimum barcode length as 300, excluding primers; ‘-L 600’ specified the maximum barcode length as 600, excluding primers. ‘-e 0’ specified the maximum number of mismatches allowed per primer as
SSC and IR regions was 35.56%, 30.78% and 43.09%, respectively. The GC content in the LSC, rRNA genes and tRNA genes was 37.78%, 55.10% and 52.73%, respectively. The size of the LSC region was inverted between the two assemblies (Supplementary Fig. S1A). In the mitogenome assembly, we used Illumina and Nanopore reads. The assembled results were identical except that the small single-copy (SSC) region (Supplementary Fig. S4A). The master chromosome encoded 54 genes: 32 PCGs, 3 rRNAs and 21 tRNAs. The plastome was a typical circular sequence containing a large single-copy (LSC) region, a pair of identical inverted repeats (IRs) and an SSC region (Supplementary Fig. S4A).

The unitig graph of the mitogenome showed a branched polymeric structure (Supplementary Fig. S4B). Different contigs (Supplementary Fig. S4B, left side) were linked to form a master chromosome (Supplementary Fig. S4B, right side). The principle chromosome can undergo rearrangement through repeat-mediated recombination, generating chromosomes with different rearrangements, called isomers45. We manually removed non-mitochondrial nodes from the graph according to the stratified coverage depth, and the repeat paths were resolved by aligning with the Nanopore long reads. Finally, a circular mitochondrial molecule was obtained (Supplementary Fig. S4). The master chromosome encoded 54 genes: 32 PCGs, 3 rRNAs and 21 tRNAs. The quantities were consistent with those found in other Asteraceae species.

General features of the A. giraldii organelle genomes. To understand the characteristics of the mitogenome and plastome of A. giraldii, we analysed their general features. The entire length of the plastome was 78,009 bp, and it was divided into four regions: an LSC region of 82,838 bp, an SSC region of 18,316 bp and a pair of identical 24,959 bp IRs (Fig. 1A). A total of 109 unique genes were found in the A. giraldii plastome: 78 PCGs, 27 tRNA genes, and 4 rRNA genes (Supplementary Table S1). Among these genes, 19 genes (rpl16, petD, petB, trnV-UAC, trnL-UALA, trnG-UCC, atpF, rpoCl, rps16, trnK-UUU and rpl2) had one intron, and two genes (clpP, ycf3) had two introns (Supplementary Table S2). Eleven cis-splicing genes (rpl16, petD, petB, clpP, ycf3, atpF, rpoCl, rps16, rpl2, ndhB and ndhA) were found in the A. giraldii plastome (Supplementary Fig. S5), and all these genes were PCGs. The cis-splicing genes rpl2 and ndhB were introns. rps12 was the only trans-splicing gene identified (Supplementary Fig. S6).

The total length of PCGs in A. giraldii plastome was 78,009 bp, representing 51.64% of the whole length of the plastome sequence. By contrast, the size of the rRNA was 9046 bp, and the size of the tRNA was 2693 bp, representing 5.99% and 1.78% of the total length of the A. giraldii plastome sequence, respectively. The GC content analysis showed that the overall GC content was 37.47%. In particular, the GC content for the protein-coding regions, rRNA genes and tRNA genes was 37.78%, 55.10% and 52.73%, respectively. The GC content in the LSC, SSC and IR regions was 35.56%, 30.78% and 43.09%, respectively.

Hypervariable region analysis. To identify the hypervariable regions among the 18 Artemisia species, we wrote a custom script to extract the intergenic spacer regions (IGS) from the GenBank files of the 18 plastomes. Firstly, we extracted the IGS sequences using extractseq. Then, we aligned the extracted sequences using clustalw273 with options ‘-type = DNA -gapopen = 10 -gapext = 2’. Finally, we calculated the genetic distance of the mitogenome and plastome of A. giraldii organelle genomes.
The total length of the *A. giraldii* mitogenome was 194,298 bp. The base composition of the entire mitogenome was A (27.26%), G (22.75%), T (27.08%) and C (22.90%). The entire GC content was 45.66%. We annotated

Figure 1. The circular maps of the organelle genomes of *A. giraldii*. (A) The circular map of the plastome. (B) The circular map of the mitogenome. The functions of the different colored genes on the map are shown on the left. The dark gray region in the inner circle indicates the GC content. The circular maps of two organelle genomes were drawn by Geseq (https://chlorobox.mpimp-golm.mpg.de/geseq.html).
A. giraldii the smallest was from the enomes ranged from 194,298 to 363,324 bp. The largest mitogenome was from the A. giraldii linearity between in D. hartwegii mitogenomes. The number of genes ranged from 24 in H. annuus to 35 similar in terms of size, ranging from 44.89 to 45.66%. Meanwhile, we collated the number of PCGs in the 10 santhemum the synteny fragments were smaller.

Species Mitogenome size (bp) Plastome size (bp) Size difference GC content (%) Number of PCGs
Lactuca sativa 363,324 152,765 210,559 45.35 32
Diplostephium hartwegii 277,718 151,994 125,724 44.89 35
Chrysanthemum boreale 211,002 151,012 59,990 45.36 35
Chrysanthemum indicum 208,097 150,972 57,125 45.41 33
Artemisia giralldi 194,298 151,072 43,226 45.66 32
Ageratum conyzoides 219,198 151,325 67,873 45.4 30
Helianthus grosseserratus 273,543 151,017 122,526 45.05 31
Helianthus annuus 300,945 151,104 149,841 45.05 27
Helianthus tuberosus 281,287 151,044 130,240 45.21 32
Helianthus strumosus 281,056 151,044 130,012 45.37 32

Table 1. Gene composition in the A. giraldii mitogenome. *” genes that contain introns.

Comparison of mitogenome and plastome in terms of size, GC content and number of PCGs in 10 Asteraceae plants.

32 PCGs in the mitogenome (Fig. 1B). According to these functions, these 32 genes can be divided into 10 classes: ATP synthase (atp1, atp4, atp6, atp8 and atp9), cytochrome (ccmB, ccmC, ccmF and ccmFn), ubiquinol cytochrome c reductase (Cob), cytochrome c oxidase (cox1, cox2 and cox3), maturases (matR), transport membrane protein (mitB), NADH dehydrogenase (nad1*, nad2*, nad3, nad4*, nad4L, nad5*, nad6, nad7*, nad9), large subunit of ribosome (rpl5, rpl10), small subunit of ribosome (rps1, rps3*, rps4, rps12, rps13) and succinate dehydrogenase (sdh4). Table 1).

Comparison of genomic features with the other nine Asteraceae mitogenomes. Angiosperm mitogenomes vary greatly in genome structure, gene content and constitution. Variations in mitogenome size can be explained mostly by difference in length among intergenic regions. We compared the length, GC content and PCG number of A. giraldii with the mitogenomes from nine other published Asteraceae species: Lactuca sativa, Diplostephium hartwegii, Chrysanthemum boreale, C. indicum, Ageratum conyzoides, Helianthus grosseserratus, Helianthus annuus, Helianthus tuberosus and Helianthus strumosus (Table 2). The length of these 10 mitogenomes ranged from 194,298 to 363,324 bp. The largest mitogenome was from the L. sativa (363,324 bp), and the smallest was from the A. giralldi (194,298 bp) in this study. The length of A. giralldi was similar to two Chrysanthemum species, and they were all relatively small in the Asteraceae species. The GC content was relatively similar in terms of size, ranging from 44.89 to 45.66%. Meanwhile, we collated the number of PCGs in the 10 mitogenomes. The number of genes ranged from 24 in H. annuus to 35 in D. hartwegii. We determined the colinearity between A. giralldi and nine Asteraceae species by using the MAFFT (v7) online service (https://mafft.cbrc.jp/alignment/server/) to identify rearrangement among them. Using A. giralldi as a reference, dotplot analysis showed synteny fragment across all species (Fig. 2). Compared with the other seven Asteraceae species, C. indicum and C. boreale had larger synteny fragments. The largest fragments were about 27 kb in C. indicum and 42 kb in C. boreale. However, compared with the synteny fragments of the other seven Asteraceae species, the synteny fragments were smaller.

Repeat sequence analysis. In addition to difference in intergenic region, diversity in mitogenome size can be attributed to a large number of repeat sequences and foreign fragments. Therefore, we analysed three
common types of repeated sequences. Microsatellites (simple repeat sequences, SSRs), also called tandem repeats of 1–6 bp, are abundant in the genomes of higher organisms and usually show high levels of polymorphism. Therefore, they are generally used as molecular markers for identifying similar species. SSRs can be classified into different types according to repeat unit. For instance, SSRs are classified into mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats according to the length of their major repeat units. We identified 36 SSRs in the plastid sequence and 51 SSRs in the mitochondrial sequence (Fig. 3, Supplementary Tables S3, S4). The most abundant SSRs in the plastome were single-nucleotide SSRs, including 19(A) and 12(T), accounting for 79.49% of the total SSRs. However, the SSRs in the *A. giraldii* mitogenome were dominated by tetranucleotide polymers, which accounted for 43.14% of all repeats. The types of SSRs in the mitogenomes were more evenly represented than in the plastomes.

Tandem repeat sequences exist in the DNA of all organisms whose genomes have been sequenced. These sequences consist of multiple contiguous repeat units and exhibit extremely high mutation rates in eukaryotes and prokaryotes because they tend to gain or lose repeat units. We identified 23 tandem repeats in the plastome and 15 in the mitogenome (Supplementary Tables S5, S6). The repeats can be further tested for their suitability as DNA fingerprinting markers.

**Figure 2.** The dotplot graphs of collinearity between the mitogenomes of the *A. giraldii* and nine Asteraceae species. The vertical axis represents the *A. giraldii* mitogenome. The horizontal axis represents the nine Asteraceae mitogenomes, respectively. The red and blue lines showed the homologous regions in the forward and reverse direction between the *A. giraldii* and nine Asteraceae species, respectively. These dotplot graphs were drawn by MAFFT online service (https://mafft.cbrc.jp/alignment/server/).
In the *A. giraldii* plastome, we identified 38 dispersed repeats: 18 forward repeats, 19 palindromic repeats, and 1 reverse repeat (Supplementary Table S7). All the dispersed repeats in the plastome were less than 100 bp, the longest was 60 bp and the shortest was 30 bp. However, the number of dispersed repeats in the mitogenome was larger than those in the plastome. In the mitogenome, we found 135 dispersed repeats comprising 85 forward repeats, 49 palindromic repeats, and 1 reverse repeat. They accounted for 62.96%, 36.30%, and 0.74% of all dispersed repeats, respectively (Supplementary Table S8). The length of the dispersed repeat sequences ranged from 30 to 248 bp, but only 17 were longer than 100 bp.

**Analysis of homologous sequences between two organelles.** The transfer of mitochondrial and plastid DNAs to the nucleus has been considered a part of the ongoing genome evolution and influences eukaryote evolution. This process not only occurs from the organelle to the nucleus but also from the plastid DNA to the mitochondrial DNA. For example, the plastid gene *rbcL* is transferred to the mitogenome numerous times during angiosperm evolution, and all evaluated sequences are pseudogenes. To investigate whether plastid DNA is transferred to mitochondrial DNA, we used BLASTN to identify potential homologous sequences between the plastome and mitogenome in *A. giraldii*, and the cutoff e-value was 1e-05. Nine DNA fragments were found between two organelle genomes (Fig. 4, Supplementary Table S9). The total length of the nine fragments was 4806 bp and accounted for 2.47% of the whole mitogenome. The longest fragment was 888 bp in the mitogenome, and the shortest was 79 bp. The location of the nine homologous fragments in the mitochondrial and plastid genomes is shown in Supplementary Table S9.

**Phylogenetic inference analysis.** We constructed phylogenetic trees with the concatenated PCG sequences, using the maximum likelihood (ML) and BI methods (Fig. 5). The phylogenetic trees constructed with plastome and mitogenome sequences had minor differences in topological structures. In both trees, the 12 species were first divided into two main clades: a large clade composed of 10 Asteraceae species and a small clade composed of two outgroup species. *A. giraldii* was closely related to *C. indicum* and *C. boreale* in the two trees. In the mitochondrial genome tree, *H. grosseserratus* and *H. annuus* were clustered on one branch, and *H. strumosus* and *H. tuberosus* was clustered on another branch. However, in the plastome tree, *H. annuus* and *H. tuberosus* were separated into different branches, whereas *H. grosseserratus* and *H. strumosus* were clustered in a clade. The second difference was that *L. sativa* was located in different positions in the two trees. In the plastid tree, *L. sativa* was located in the outermost clade formed by the Asteraceae family. In the mitochondrial tree, *L. sativa* was located within the clade formed by the Asteraceae species (Fig. 5).
Selective pressure analysis of *A. giraldii* mitogenomic genes. To determine which genes are subject to positive selection, we calculated the LRT p-value based on the lnL and np values of the null and alternative models for 28 protein-coding genes in the mitogenome. Then the likelihood ratio test (LRT) p-values were adjusted (Supplementary Table S10). The detailed analysis results can be found in Supplementary Table S11. The adjusted p-value of *ccmFc*, *nad1*, *nad6*, *atp9*, *atp1* and *rps12* is below 0.05, suggesting these six genes are subject to positive selection.

Molecular marker development. Based on the 18 plastome sequences of *Artemisia* species, we found one molecular marker for distinguishing among 18 *Artemisia* species (Supplementary Table S12). It was a pair of highly conserved regions that can be used for primer design. The regions amplified by the primer pairs contained one or more SNP and INDEL sites that can be used in distinguishing among the 18 *Artemisia* species. However, the lengths of the regions were about 30 kb, which is extremely long for practical uses.

Analysis of hypervariable regions. A total of 14 IGS were hypervariable regions (Fig. 6). The top three regions: *ndhG-ndhI*, *ccsA-ndhD* and *rpl32-trnL-UAG* had K2p values of 1.50, 1.22 and 1.06, respectively. We first extracted the top three hypervariable regions and aligned them (Supplementary Fig. S7). However, the only two variant sites in *ccsA-ndhD* regions also existed in *rpl32-trnL-UAG* regions. Hence, we selected two regions: *ndhG-ndhI* and *rpl32-trnL-UAG* for molecular marker development. The variant sites in the two hypervariable regions can be used in distinguishing among the 18 species completely, including 11 SNPs and six indel sites (Supplementary Fig. S7). As indicated in Supplementary Fig. S7A, SNP 1–6 can be used in distinguishing among *Artemisia hallaisanensis*, *Artemisia absinthium* var. calcigena, *Artemisia frigida*, *Artemisia maritima*, *Artemisia argyi* and *Artemisia fukudo* with other 17 species. Indel 1–3 can be used in discriminating among *Artemisia freyniana*, *Artemisia lactiflora* and *Artemisia gmelinii*. As demonstrated in Supplementary Fig. S7B, SNP7-11 can be used in identifying *A. frigida*, *Artemisia capillaris*, *Artemisia stolonifera*, *Artemisia montana* and *Artemisia scoparia*. Indel 4 and indel 5 can be used in identifying *Artemisia selengensis* and *A. annua* with other 17 species. After distinguishing among above 16 species, the remaining two species, *Artemisia ordosica* and *Artemisia tangutica* can be distinguished from each other by using indel 6.
**Discussion**

*Artemisia giraldii* is a medicinal plant primarily used as a source of traditional medicines. Obtaining its genomic information is the critical step for understanding the biosynthesis of its active components. As the first step, we sequenced and assembled the mitogenome and plastome of *A. giraldii* in the current study. Then, we analysed the mitogenome and plastome's general features and compared them in detail.

In the plastome, two copies of IRs separate SSC and LSC regions. When an IR region is present, homologous recombination occurs between the two copies and results in the frequent 'flip' inversion of the SSC region between the two copies, thus allowing two heterogeneous genomic orientations to coexist in a single plant with approximately the same frequency.

In this study, we used two strategies to assemble the plastome of *A. giraldii*. The two strategies generated two assemblies that were identical, except that the SSC region was inverted (Supplementary Fig. S1A). The reverse and complement of the SSC region in the plastome assembly from Illumina and Nanopore data generated an assembly identical to that assembled by Illumina data (Supplementary Fig. S1B). Coverage depth is an indicator used in evaluating the correctness of an assembly in the mitochondria and the plastid genome assembling process. The drop of coverage depth is often considered a sign of misassembly. We observed several regions with low depths (Supplementary Figs. S2A and S3A,B). To determine whether assembling problems occurred, we visually examined the regions. The mapped results (Supplementary Figs. S2B and S3C) showed the reads sufficiently covered the regions, suggesting that the regions were correctly assembled. Further examination showed that the regions were AT rich. The AT-rich regions tend to be highly polymorphic and are error prone for long-read sequencing and result in a low coverage depth.

The mitogenome of plants is much larger than the plastome because of frequent exchange with nuclear and chloroplast DNA, repeat sequences, AT-rich non-coding regions, large introns and non-coding sequences. The mitogenome size commonly ranges from 200 to 2400 kb in angiosperms. By contrast, the plastome size commonly ranges from 100 to 200 kb. We compared the sizes of the mitogenomes and plastomes of plants released in GenBank to determine if the small difference between the two organelles is unusual. Our results showed a small difference in size between the mitogenome and plastome in *A. giraldii* among the 318 species having both mitogenomes and plastomes released in GenBank by August 1st, 2022 (Supplementary Table S13).

The size difference between the mitochondria and plastid genomes in *A. giraldii* was extremely small, only 43,226 bp, compared with the size difference in other species. Among the 318 species, 95 showed the smaller difference between mitogenome and plastome sizes than *A. giraldii*. 94 of the 95 species were algae and mosses. The only angiosperm plant having a smaller size difference was *Bidens pilosa* from Asteraceae, with 1236 bp.
Actually, its size difference was the smallest among all pairs of mitogenomes and plastomes in this study. These observations suggested that mitogenome expansion develops along with plant evolution.

Among the Asteraceae species, *A. giraldii* had the second smallest difference. The other seven Asteraceae species, *Bidens parviflora, Bidens biteminata, Bidens bipinnata, Chrysanthemum indicum, Chrysanthemum boreale, Bidens tripartite,* and *Ageratum conyzoides,* also had small size differences between their two organelle genomes, which were 44,511, 46,989, 46,990, 57,125, 59,990, 66,297 and 67,873 bp, respectively. This result indicated that small size difference is a common phenomenon in Asteraceae. The cause of this phenomenon has not yet been reported, and thus the specific mechanisms need to be further explored.

We drew a figure to show the sizes of the seven most representative mitogenomes. The largest known mitogenome was obtained from *Cucumis melo.* The smallest known angiosperm mitogenome was obtained from *Bidens pilosa.* The sizes of four Asteraceae mitogenomes were in between (Fig. 7). The mitogenomes of different plants differ greatly in size.

We analysed the homologous sequence between mitogenome and plastome. Sequence migration is common in plants. The plastid or nuclear DNA fragments can be inserted into mitochondrial DNA, resulting in an expanding mitogenome. These cp-derived mtDNAs can contain complete or partial PCG sequences and some tRNA sequences. Frequently, these transfer sequences have no functions. We found nine homologous fragments between the plastid DNA and mitochondrial DNA. The total length of the nine fragments was 4806 bp and accounted for 2.47% of the whole mitogenome. To determine whether these homologous sequences originated from their common ancestor (vertical transfer) or were transferred from plastid to mitochondria (horizontal transfer), we determined whether these homologous sequences were present in the plastome and mitogenome of *C. boreale* with BLASTN. We found homologous sequences for eight fragments: F1, F2, F3, F5, F6, F7, F8 and F9 (Supplementary Table S9) in the plastome and mitogenome of *C. boreale.* We only found a homologous sequence for fragment F4 in the plastome of *C. boreale.* Therefore, we speculated that eight homologous fragments (F1, F2, F3, F5, F6, F7, F8 and F9) may have originated from their common ancestor (vertical transfer) or were transferred from plastid to mitochondria (horizontal transfer), but we determined whether these homologous sequences were present in the plastome and mitogenome of *C. boreale* with BLASTN. We found homologous sequences for eight fragments: F1, F2, F3, F5, F6, F7, F8 and F9 (Supplementary Table S9) in the plastome and mitogenome of *C. boreale.* We only found a homologous sequence for fragment F4 in the plastome of *C. boreale.* Therefore, we speculated that eight homologous fragments (F1, F2, F3, F5, F6, F7, F8 and F9) may have originated from their common ancestor and have been preserved throughout evolution. Another homologous fragment (F4) may have been transferred from the plastome to the plastid DNA in *A. giraldii.* Thus, we suspected that a low degree of DNA exchange between the mitochondria and plastid DNAs is responsible for the low level of mitogenome expansion in *A. giraldii.*

Compared with plastomes and nuclear genes, the mitogenome has been rarely used in reconstruct phylogenies partly because of the slower nucleotide substitution rate and the difficulty of complete assembly and direct alignment. We used the sequences of common genes to construct mitochondrial and plastid trees with ML and BI methods. *A. giraldii* was placed in the same locations in both trees. However, the plastid and mitochondrial trees differed in topology, particularly in the branch containing *L. sativa* and four *Helianthus* species. In the
plastid, the \textit{L. sativa} was located in the outermost clade formed by the Asteraceae family. In the mitochondrial tree, \textit{L. sativa} was located within the clade formed by the Asteraceae species. Hence, the plastid tree was more in line with the taxonomic classification compared with the mitochondrial tree. \textit{L. sativa} and \textit{Asteroideae} species are located in different branches of the phylogenetic tree\textsuperscript{100,101}. To further understand the relationship of mitochondrial genomes among 10 Asteraceae species, we aligned the mitogenome of \textit{A. giraldii} (NC_064134.1) by using the BLASTN suite in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The results showed that the sequence similarity between 10 Asteraceae species was consistent with those shown in the mitochondrial tree (Supplementary Table S14). Compared with the four \textit{Helianthus} species and \textit{A. conyzoides}, the sequence similarity between \textit{A. giraldii} and \textit{L. sativa} was higher.

Previous report and sequence alignment results confirm the incongruence between the plastome tree and mitogenome tree for \textit{L. sativa}. We hypothesised that the difference in topology between the two trees results from the inconsistent evolutionary rates of the plastome and mitogenome. Further analysis of the mitogenome of \textit{L. sativa} is required to elucidate the incongruence. However, the support value between \textit{H. grosseserratus} and \textit{H. strumosus} in the plastome and between \textit{H. grosseserratus} and \textit{H. annuus} were less than 50 because of the high sequence similarity among \textit{Helianthus} species, making the branches inseparable. The \textit{A. giraldii} reported in this study had the same branch structure in the two trees and had a high support value, suggesting high credibility for its evolutionary relationship. The closest relatives to \textit{A. giraldii} were \textit{C. indicum} and \textit{C. boreale}. This result is consistent with their taxonomic relationship, as they both belong to Artemisiinae. The collinearity results confirmed this conclusion. \textit{C. indicum} and \textit{C. boreale} had a larger synteny fragment than the \textit{A. giraldii} mitogenome. Overall, the results revealed that the gene orders on the mitogenomes of the 10 Asteraceae species differed significantly.

Most mitochondrial genes are highly conserved and have undergone neutral and negative selection. The selective pressure analysis is commonly used in identifying positively or negatively selected genes to adapt to a particular lifestyle. In this analysis, the adjusted p-values of \textit{ccmFpc}, \textit{nad1}, \textit{nad6}, \textit{atp9}, \textit{atp1} and \textit{rps12} were below 0.05, suggesting that these genes underwent positive selection in the evolution process. The other 22 genes were more conserved and not subject to positive selection. The adjusted p-values of \textit{ccmFpc} and \textit{nad1} were 0, suggesting that they are subject to strong positive selection. \textit{ccmFpc} was a protein similar to the C-terminal part of the bacterial \textit{ccmF}. It is involved in cytochrome c maturation and is present in a large-sized complex in wheat mitochondria\textsuperscript{102}. \textit{nad1} is one of the NADH dehydrogenases and plays an important role in mitochondrial electron transport\textsuperscript{103}. Given the limited availability of mitogenomes in \textit{Artemisia}, we used the plastome sequences of 18 \textit{Artemisia} species to predict one pair of primers that potentially amplify a variable DNA region to distinguish among 18 \textit{Artemisia} species. However, the length of the predicted amplified fragment was extremely long to validate. We concluded that this molecular marker may not be applicable to distinguish them. Instead, we analysed the hypervariable regions of the 18 species to obtain available molecular markers. Owing to the large number of species, the variant site in one hypervariable region cannot be used in distinguishing 18 species from one another. The variant site in ccsA-ndhD is present in \textit{rpl32-trnl-UAG}, and thus the 17 variant sites in the two hypervariable regions (ndhG-ndhI and \textit{rpl32-trnl-UAG}) were combined (11 SNPs and six indels). We were able
to completely distinguish among 18 Artemisia species (Supplementary Fig. S7). Further experimental verification of these molecular markers is needed.

Conclusions
In this study, we assembled the mitogenome and plastome of A. giraldii for the first time. Phylogenetic analysis showed that the branch locations of A. giraldii in the phylogenetic trees constructed with the mitochondrial and plastid protein sequences were identical, suggesting the possible co-evolution of the genomes from the two organelles. Homologous sequence analysis identified nine homologous fragments between two organelles, and one fragment might have transferred from the plastome into the mitogenome. This study may provide a reference for studying the evolutionary relationship between mitochondria and plastids in Asteraceae species.

Data availability
The plastome and mitogenome sequences of A. giraldii reported in this article are available in GenBank (https://www.ncbi.nlm.nih.gov/) with accession numbers OK128342 and NC_064134.1, respectively. The raw data have been submitted to the SRA database (BioSample: SRR25050459; BioProject: PRJNA798221; SRA: SRR17652243). The sample has been deposited in the Institute of Medicinal Plant Development (Beijing, China) with accession number implad201910017.

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References
1. Bremer, K. & Humphries, C. J. Generic monograph of the Asteraceae-Artemiaceae. Bull. Nat. Hist. Museum Bot. Ser. 23, 71–177 (1993).
2. Martín, J., Torrell, M., Korobkov, A. & Vallès, J. Palynological features as a systematic marker in Artemisia L. and related genera (Asteraceae, Anthemideae)-II: Implications for Subtribe Artemisiasinae delimitation. Plant Biol. 5, 85–93 (2003).
3. Watson, L. E., Bates, P. L., Evans, T. M., Unwin, M. M. & Estes, J. R. Molecular phylogeny of subtribe Artemisiasinae (Asteraceae), including Artemisia and its allied and segregate genera. BMC Evol. Biol. 2, 1–12 (2002).
4. Irschick, M., Emami, S. A. & Mahmoud, S. M. Detection of sesquiterpene lactones in ten Artemisia species population of Khorasan provinces. (2007).
5. Abad, M. J., Bedoya, L. M., Apaza, L. & Bermejo, P. The Artemisia L. genus: A review of bioactive essential oils. Molecules 17, 2542–2566 (2012).
6. Koul, B., Taak, P., Kumar, A., Khatri, T. & Sanyal, I. The Artemisia genus: A review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. J. Glycosides Lipidomics 7, 1–7 (2018).
7. Zheng, W., Tan, R., Yang, L. & Liu, Z. A new antimicrobial sesquiterpene lactone from Artemisia giraldii. Spectrosc. Lett. 29, 1589–1597 (1996).
8. Zheng, W., Tan, R., Yang, L. & Liu, Z. Two flavones from Artemisia giraldii and their antimicrobial activity. Planta Med. 62, 160–162 (1996).
9. Obistuón, D. et al. Chemical characterization by GC-MS and in vitro activity against Candida albicans of volatile fractions prepared from Artemisia dracunculus, Artemisia abrotanum, Artemisia absinthium and Artemisia vulgaris. Chem. Cent. J. 8, 1–11 (2014).
10. Shafi, G. et al. Artemisia absinthium (AA): A novel potential complementary and alternative medicine for breast cancer. Mol. Biol. Rep. 39, 7373–7379 (2012).
11. Mojarrah, M., Emami, S., Gheibi, S., Taleb, A. & Heshmati Afshar, F. Evaluation of anti-malarial activity of Artemisia turcomanica and A. kopetdaghensis by cell-free β-hematin formation assay. Res. J. Pharmacogn. 3, 59–65 (2016).
12. Taherkhani, M. In vitro cytotoxic activity of the essential oil extracted from Artemisia absinthium. Iran. J. Toxicol. 8, 1152–1156 (2014).
13. Altunkaya, A., Yildirm, B., Ekici, K. & Terzioglu, O. Determining essential oil composition, antibacterial and antioxidant activity of water wormwood extracts. (2018).
14. Rajeshkumar, P. & Hosagoudar, V. Mycorrhizal fungi of Artemisia L. and related genera (Asteraceae, Anthemideae)-II: Implications for Subtribe Artemisiasinae delimitation. Plant Biol. 5, 71–84 (2003).
15. Klayman, D. L. Qinghaosu (artemisinin): An antimalarial drug from China. Nat. Med. 4, 128–132 (1998).
16. Tu, Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat. Med. 17, 1217–1220 (2011).
17. Liu, F., Yang, J. & Zhang, P. Relationships between geographical distribution of Artemisia giraldii and climatic factors. J. Arid Land Resour. Environ. 26, 56–59 (2012).
18. Zhicheng, Z., Donglin, J., Ming, H. Preliminary study on the biomass of Artemisia giraldii community. Grassland China 5, 6–13 (1997).
19. Tan, R. et al. Mono- and sesquiterpenes and antifungal constituents from Artemisia species. Planta Med. 65, 064–067 (1999).
20. Zheng, W., Tan, R. & Liu, Z. A study of terpenoids in petrol extracts of eight Artemisia species. J. Nanjing Univ. Nat. Sci. Ed. 32, 706–712 (1996).
21. Chu, S.-S., Liu, Z.-L., Du, S.-S. & Deng, Z.-W. Chemical composition and insecticidal activity against Sitophilus zeamais of the essential oils derived from Artemisia dracunculus and Artemisia absinthium. J. Appl. Entomol. 137, 725–7265 (2012).
22. Gray, M. W. The endosymbiont hypothesis revisited. Int. Rev. Cytol. 141, 233–357 (1992).
23. Hikosaka, K. et al. Divergence of the mitochondrial genome structure in the apicomplexan parasites, Babesia and Theileria. Mol. Biol. Evol. 27, 1107–1116 (2010).
24. Smith, D. R. & Keeling, P. J. Mitochondrial and plastid genome architecture: Reoccurring themes, but significant differences at the extremes. Proc. Natl. Acad. Sci. 112, 10177–10184 (2015).
25. Mower, J. P., Sloan, D. B. & Alverson, A. J. Plant mitochondrial genome diversity: The genomics revolution. Plant Genome Divers. 1, 123–144 (2012).
26. Shtratnikova, V. Y., Shchelkunov, M. I., Penin, A. A. & Logacheva, M. D. Mitochondrial genome of the nonphotosynthetic myco-heterotrophic plant Hypopitys monophora, its structure, gene expression and RNA editing. PeerJ 8, e9309 (2020).
27. Yu, R. et al. The minicircular and extremely heteroplasmic mitogenome of the holoparasitic plant Rhopalocnemis phalloides. Curr. Biol. 32, e70–e79 (2022).
28. Yudina, S. V. et al. Comparative analysis of plastid genomes in the non-photosynthetic genus Thismia reveals ongoing gene set reduction. Front. Plant Sci. 12, 602598 (2021).
29. Smith, D. R. et al. The Dunaliella salina organelle genomes: Large sequences, inflated with intronic and intergenic DNA. BMC Plant Biol. 10, 1–14 (2010).
30. Ladoukakis, E. D. & Zouros, E. Evolution and inheritance of animal mitochondrial DNA: Rules and exceptions. *J. Biol. Res.-Thessaloniki* 24, 1–7 (2017).

31. Boore, J. L. Animal mitochondrial genomes. *Nucleic Acids Res.* 27, 1767–1780 (1999).

32. Quetier, F. & Vedel, F. Heterogeneous population of mitochondrial DNA molecules in higher plants. *Nature* 268, 365–368 (1977).

33. Bendich, A. J. Reaching for the ring: The study of mitochondrial genome structure. *Carr. Genet.* 24, 279–290 (1993).

34. Sloan, D. B. One ring to rule them all? Genome sequencing provides new insights into the ‘master circle’ model of plant mitochondrial DNA structure. *New Phytol.* 200, 978–985 (2013).

35. Adams, K. L., Qiu, Y.-L., Stoutemyer, M. & Palmer, J. D. Punctuated evolution of mitochondrial gene content: High and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proc. Natl. Acad. Sci. USA* 99, 9905–9912 (2002).

36. Adams, K. L. & Palmer, J. D. Evolution of mitochondrial gene content: Gene loss and transfer to the nucleus. *Mol. Phylogenet. Evol.* 29, 380–395 (2003).

37. Palmer, J. D. & Shields, C. R. Tripartite structure of the plastid genome. *Proc. Natl. Acad. Sci. USA* 97, 9249–9261 (1994).

38. Mower, J. P., Case, A. L., Floro, E. R. & Willis, J. H. Evidence against equimolarity of large repeat arrangements and a predominant master circle structure of the mitochondrial genome from a monkeyflower (*Mimulus guttatus*) lineage with cryptic CMS. *Genome Biol. Evol.* 4, 670–686 (2012).

39. Kozik, A. et al. The alternative reality of plant mitochondrial DNA: One ring does not rule them all. *PLoS Genet.* 15, e1008373 (2019).

40. Maréchal, A. & Brisson, N. Recombination and the maintenance of plant organelle genome stability. *New Phytol.* 186, 299–317 (2010).

41. Mackenzie, S. A. The unique biology of mitochondrial genome instability in plants. *Plant Mitochondria*. 36 (2007).

42. Alversen, A. J. et al. Insights into the evolution of mitochondrial genome size from complete sequences of *Citrullus lanatus* and *Cucurbita pepo* (Cucurbitaceae). *Mol. Biol. Evol.* 27, 1436–1448 (2010).

43. Ogihara, Y. et al. Structural dynamics of cereal mitochondrial genomes as revealed by complete nucleotide sequencing of the wheat mitochondrial genome. *Nucleic Acids Res.* 33, 6235–6250 (2005).

44. Kempken, F. & Pring, D. Progress in Botany 139–166 (Springer, 1999).

45. Bolger, A. M., Lahse, M. & Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120 (2014).

46. Jin, J.-J. et al. GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol.* 21, 1–31 (2020).

47. Li, H. Minimap and miniasm: Fast mapping and de novo assembly for noisy long sequences. *Bioinformatics* 32, 2103–2110 (2016).

48. Shi, L. et al. CPGAVAS2, an integrated plastome sequence annotator and analyzer. *Nucleic Acids Res.* 47, W65–W73 (2019).

49. Xia, Y. et al. The complete chloroplast genome sequence of *Chrysanthemum indicum*. *Mitochondrial DNA Part A* 27, 4688–4689 (2016).

50. Tillich, M. et al. GeSeq–versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 45, W6–W11 (2017).

51. Wang, S. et al. Assembly of a complete mitogenome of *Chrysanthemum nankingense* using Oxford Nanopore long reads and the diversity and evolution of Asteraceae mitogenomes. *Genes* 9, 547 (2018).

52. Chan, P. P. & Lowe, T. M. Gene Prediction 1–14 (Springer, 2019).

53. Lewis, S. E. et al. Apollo: A sequence annotation editor. *Genome Biol.* 3, 1–14 (2002).

54. Greiner, S., Lehwalk, P. & Bock, R. OrganellarGenomesDRAW (OGDRAW) version 1.3.1: Expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res.* 47, W59–W64 (2019).

55. Chen, Y., Ye, W., Zhang, Y. & Xu, Y. High speed BLASTN: An accelerated MegaBLAST search tool. *Nucleic Acids Res.* 43, 7762–7768 (2015).

56. Zhang, H., Melzer, P. & Davis, S. RCircos: An R package for Circos 2D track plots. *BMC Bioinform.* 14, 1–5 (2013).

57. Chen, C. et al. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13, 1194–1202 (2020).

58. Reier, S., Thiel, T., Münch, T., Scholz, U. & Mascher, M. MISA-web: A web server for microsatellite prediction. *Biosci. Biotechnol. Biochem.* 73, 2583–2585 (2017).

59. B sanitizer, G. Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Res.* 27, 573–580 (1999).

60. Kurtz, S. et al. REPuter: The manifold applications of repeat analysis on a genomic scale. *Nucleic Acids Res.* 29, 4633–4642 (2001).

61. Zhang, D. et al. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour.* 20, 348–355 (2020).

62. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780 (2013).

63. Castresana, J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552 (2000).

64. Minh, B. Q. et al. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534 (2020).

65. Ivica, L. & Peer, R. Interactive Tree Of life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 49(W1), W293–W296 (2021).

66. Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. *ModellTest* 2: More models, new heuristics and parallel computing. *Nat. Methods* 9, 772–772 (2012).

67. Ronquist, F. et al. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542 (2012).

68. Gao, F. et al. EasyCodeML: A visual tool for analysis of selection using Codelml. *Ecol. Evol.* 9, 3891–3898 (2019).

69. Yang, Z. & Nielsen, R. Codon-substitution models for detecting molecular adaptation at individual sites along specific lineages. *Mol. Biol. Evol.* 19, 908–917 (2002).

70. Thissen, D., Steinberg, L. & Kuang, D. Quick and easy implementation of the Benjamini–Hochberg procedure for controlling the false positive rate in multiple comparisons. *J. Educ. Behav. Stat.* 27, 77–83 (2002).

71. Tsay, R. et al. ecoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Res.* 39, e145 (2011).

72. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948 (2007).

73. Rice, P., Longden, I. & Bleasby, A. EMBOSS: The European molecular biology open software suite. *Trends Genet.* 16, 276–277 (2000).

74. Milne, I. et al. Using Tablet for visual exploration of second-generation sequencing data. *Brief. Bioinform.* 14, 193–202 (2013).

75. Wick, R. R., Schultz, M. B., Zobel, J. & Holt, K. E. Bandage: Interactive visualization of de novo genome assemblies. *Bioinformatics* 31, 3350–3352 (2015).
77. Katoh, K., Rozewicki, J. & Yamada, K.-D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinform. 20, 1160–1166 (2019).
78. Park, S. et al. Dynamic evolution of Geranium mitochondrial genomes through multiple horizontal and intracellular gene transfers. New Phytol. 208, 570–583 (2015).
79. Ellegren, H. Microsatellites: Simple sequences with complex evolution. Nat. Rev. Genet. 5, 435–445 (2004).
80. Guang, X.-M. et al. IDSSR: An efficient pipeline for identifying polymorphic microsatellites from a single genome sequence. Int. J. Mol. Sci. 20, 3497 (2019).
81. Fan, H. & Chu, J.-Y. A brief review of short tandem repeat mutation. Genomics Proteomics Bioinform. 5, 7–14 (2007).
82. Bichara, M., Wagner, I. & Lambert, I. Mechanisms of tandem repeat instability in bacteria. Mutat. Res. Fundam. Mol. Mech. Mutagen. 598, 144–163 (2006).
83. Richly, E. & Leister, D. NUMTs in sequenced eukaryotic genomes. Mol. Biol. Evol. 21, 1081–1084 (2004).
84. Huang, C.-Y., Grunheit, N., Ahmadinejad, N., Timmis, J. N. & Martin, W. Mutational decay and age of chloroplast and mitochondrial genomes transferred recently to angiosperm nuclear chromosomes. Plant Physiol. 138, 1723–1733 (2005).
85. Sugiyama, Y. et al. The complete nucleotide sequence and multiparticle organization of the tobacco mitochondrial genome: Comparative analysis of mitochondrial genomes in higher plants. Mol. Genet. Genomics 272, 603–615 (2005).
86. Notsu, Y. et al. The complete sequence of the rice (Oryza sativa L.) mitochondrial genome: Frequent DNA sequence acquisition and loss during the evolution of flowering plants. Mol. Genet. Genomics 268, 434–445 (2002).
87. Cummings, M. P., Nugent, J. M., Olmstead, R. G. & Palmer, J. D. Phylogenetic analysis reveals five independent transfers of the chloroplast gene rbcL to the mitochondrial genome in angiosperms. Curr. Genet. 43, 131–138 (2003).
88. Knox, E. B. The dynamic history of plastid genomes in the Campanulaceae sensu lato is unique among angiosperms. Proc. Natl. Acad. Sci. 111, 11097–11102 (2014).
89. Palmer, J. D. Chloroplast DNA exists in two orientations. Nature 301, 92–93 (1983).
90. Stein, D. B., Palmer, J. D. & Thompson, W. F. Structural evolution and flip-flop recombination of chloroplast DNA in the fern genus Osmunda. Curr. Genet. 10, 835–841 (1986).
91. Delahaye, C. & Nicolas, J. Sequencing DNA with nanopores: Troubles and biases. PLoS ONE 16, e0257521 (2021).
92. Dong, S. et al. The complete mitochondrial genome of the early flowering plant Nymphaea colorata is highly repetitive with low recombination. BMC Genomics 19, 1–12 (2018).
93. Timmis, J. N., Ayliffe, M. A., Huang, C. Y. & Martin, W. Endosymbiotic gene transfer: Organelle genomes forge eukaryotic chromosomes. Nat. Rev. Genet. 5, 123–135 (2004).
94. Unseld, M., Marienfeld, J. R., Brandl, P. & Brennicke, A. The mitochondrial genome of Arabidopsis thaliana contains 57 genes in 366,924 nucleotides. Nat. Genet. 15, 57–61 (1997).
95. Kubo, T. & Newton, K. J. Angiosperm mitochondrial genomes and mutations. Mitochondrion 8, 5–14 (2008).
96. Wang, X.-C., Chen, H., Yang, D. & Liu, C. Diversity of mitochondrial plastid DNAs (MTPTs) in seed plants. Mitochondrial DNA Part A 29, 635–642 (2018).
97. Clifton, S. W. et al. Sequence and comparative analysis of the maize Nb mitochondrial genome. Plant Physiol. 136, 3486–3500 (2004).
98. Van de Paer, C., Bouchez, O. & Besnard, G. Prospects on the evolutionary mitogenomics of plants: A case study on the olive family (Oleaceae). Mol. Ecol. Resour. 18, 407–423 (2018).
99. Vargas, O. M., Ortiz, E. M. & Simpson, B. B. Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: Diplostephium). New Phytol. 214, 1736–1750 (2017).
100. Kim, J.-K. et al. The complete chloroplast genome sequence of the Taraxacum officinale FH Wigg (Asteraceae). Mitochondrial DNA Part B 1, 228–229 (2016).
101. Walker, L. E., Zanis, M. I. & Emery, N. C. Comparative analysis of complete chloroplast genome sequence and inversion variation in Lasthenia burkei (Madieae, Asteraceae). Am. J. Bot. 101, 722–729 (2014).
102. Giege, P., Rayapuram, N., Meyer, E. H., Grienenberger, J. M. & Bonnard, G. CcmFC involved in cytochrome c maturation is present in a large sized complex in wheat mitochondria. FEBS Lett. 563, 165–169 (2004).
103. Weiss, H., Friedrich, T., Hofhaus, G. & Preis, D. The respiratory-chain NADH dehydrogenase (complex I) of mitochondria. EJB Rev. 1991, 55–68 (1991).

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Author contributions
C.L. conceived the study; Y.N. assembled and annotated the mitogenome and plastome; J.Y. collated the data; J.Y. and Q.L. carried out the comparative analysis; J.Y. wrote the manuscript; C.L. and P.C. reviewed the manuscript critically. All authors read and approved the manuscript.

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Competing interests
The authors declare no competing interests.

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