Comparative analysis of mitochondrial genome between two flower color of Chrysanthemum indicum

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Abstract

Chrysanthemum indicum is a perennial herb that is valued for its multifaceted medicinal and ornamental properties. Intraspecific variation in floral traits, most notably flower color, is common in wild and cultivated populations. To reveal the complex evolutionary characteristics and processes of *Chrysanthemum* mitogenomes, study the intraspecific variation, we assembled complete mitochondrial genome of two diploid *C. indicum* exhibiting white and yellow corollas and compared them with the previously published organellar genomes between diploid and tetraploid *Chrysanthemum indicum*. A hybrid assembly approach that integrated Illumina short reads with PacBio HiFi long reads produced two mitogenomes of 208,791 bp and 208,787 bp (GC content 45.50%). Each genome contained 32 protein-coding genes, 17 tRNAs and three rRNAs, together with 149 pairs of repetitive sequences ≥ 30 bp. Whole-genome alignment and gene-order comparison detected no structural rearrangements or consistent single-nucleotide differences between the color variants, indicating exceptional mitochondrial sequence conservation at the intraspecific level. Phylogenetic reconstruction based on 26 core mitochondrial protein-coding genes placed both morphs within a well-supported *Chrysanthemum* clade that was congruent with current angiosperm classification systems. These high-quality, annotated mitogenomes expand the genomic resource base for *Chrysanthemum* and provide a robust reference for future investigations into the molecular evolution, cytoplasmic inheritance, germplasm authentication, and breeding of this economically important species.

Introduction

Mitochondria are critical for several aspects of plant growth and development as well as in plant energy metabolism. Plant mitochondria are the primary hubs of ATP synthesis, redox signals and stress memory, and tend to exhibit more complex structures than animal mitogenomes, which often exist in diverse forms beyond the typical circular structure[1]. Whereas chloroplast genomes conform to a conservative 120–160 kb single-circle topology, mitogenomes vary > 200-fold, from 66 kb in the parasitic mistletoe *Viscum scurruloideum*[2] to 11.7 Mb in the *Silene conica*[3], and can exist as single chromosomes, multi-chromosomal circles, linear chromosomes or branched networks generated by repeat-mediated recombination[4]. The complexity of the plant mitogenome structure, interactions with RNA, characteristics of introns, and horizontal gene transfer have emerged as important areas of research in plant molecular biology and genomics.

The maturation of Illumina short reads and long-read sequencing (PacBio HiFi/ONT) has facilitated the assembly of plant mitogenomes, including *Eriobotrya japonica*[5], *Salvia miltiorrhiza*[6], *Salvia officinalis*[7], and *Coffea arabica*[8]. Over 600 mitogenomes have been released in GenBank[9]. Mitogenomic data have greatly advanced our understanding of species origins, plant diversity, and cytoplasmic evolution. In particular, phylogenetic studies of three significant plant families, Arecaceae[10], Rosaceae[11], and Convolvulaceae[5] have been instrumental. *Chrysanthemum indicum* is a member of the Asteraceae family and is widely distributed across China, Japan, Korea, and other countries[12]. This plant has significant medicinal properties including antibacterial, antiviral[13], and anti-inflammatory activities[14].

The aqueous extract of *C. indicum* has been shown to exhibit anti-adipogenic effects, indicating its potential as a treatment for obesity[15]. Beyond pharmacology, the species underpins the ornamental economy of both Europe and Asia; it supplies > 35% of Japanese cut-flower acreage and is a key component of the United Kingdom floristry trade[16].

Previous studies have focused on the chloroplast genomes of cultivated *Chrysanthemum* species[17] or on the mitochondrial structural variation among different ploidy levels of wild *C. indicum*[18]. In this study, two mitogenomes of *C. indicum* with white and yellow corollas were sequenced based on short and long-read sequencing data. We conducted a comprehensive analysis and comparison of the two *C. indicum* mitogenomes, focusing on codon usage preferences, repetitive sequences, and gene transfer that occurs between the mitogenome and chloroplast genome. Additionally, the evolutionary relationship of two *C. indicum* and two diploid *C. indicum* were further studied, indicating that both white-rayed and yellow-rayed *C. indicum* belong to the order of Asterales, and are clustered together with species of *Chrysanthemum*. The findings from these studies will greatly contribute to future studies in the fields of molecular genetics and evolutionary research on *C. indicum*.

Materials and methods

Plant materials, DNA extraction, and sequencing

Wild *C. indicum* was collected from the germplasm repository of China Resources Sanjiu Medical & Pharmaceutical Co., Ltd. in Yangxin County, Huangshi City, Hubei Province, China. DNA was extracted using a CTAB-based protocol. The purified mtDNA was then sequenced on both the Illumina (HiSeq Xten PE150 Illumina) and Nanopore (Oxford Nanopore GridION × 5 Oxford Nanopore) platforms. All raw reads were generated by Wuhan Benagen Tech Solutions Co., Ltd.

Mitogenome assembly and annotation

The mitogenomes of the two *C. indicum* were assembled using short reads (Illumina) and long reads sequencing (Nanopore). Flye was employed to generate an initial contig set from the long reads, with default parameters, and the short reads were mapped to these contigs using BWA-MEM (v 0.7.17)[19]. The resulting alignment was supplied to Unicycler to accomplish *C. indicum* mitogenome assembly[20], and the assembled genomes were visually represented using Bandage (v 0.8.1)[21]. Geseq (v 2.03) was used to annotate the protein-coding genes (PCGs) of two *C. indicum* mitogenomes[22] using *Liriodendron tulipifera* (NC_021152.1) and *Arabidopsis thaliana* (NC_037304) as references. The two *C. indicum* mitochondrial genome tRNAs and rRNAs were predicted using tRNAscan-SE (v.2.0.11) [23]and BLASTN (v 2.13.0)[24]. Manual curation of gene annotations in the mitogenome was performed using Apollo (v. 1.11.8)[25].

Codon preference analysis

The protein-coding sequences of the two *C. indicum* mitogenomes were extracted using Phylosuite (v.1.1.16)[26]. The codon preference of PCGs was analyzed using Mega (v 7.0)[27], and Relative Synonymous Codon Usage (RSCU) was calculated[28].

Analysis of repeated sequence

The repeated sequences of two *C. indicum* mitogenomes were identified using the Microsatellite Identification tool (MISA v2.1)[29], Tandem Repeats Finder (TRF v4.09)[30], and REPuter Web Server including simple sequence repeats (SSR), tandem repeats and dispersed repeats[31].

Transformation of Chloroplast DNA into Mitochondria and analysis of RNA editing

The chloroplast genomes of two *C. indicum* were assembled and annotated using GetOrganelle (v.1.7.7.0)[32] and CPGAVAS2[32]. The *C. indicum* genome annotation results were manually adjusted using CPGView[33]. Homologous fragments were analyzed using BLASTN (v 2.13.0)[24], and the results were visualized using Circos (v 0.69-9)[34]. Deepred-mt was used to predict RNA editing events in the PCGs of the *C. indicum* mitogenome[35].

Phylogenetic analysis

The mitogenomes of 35 plant species were acquired from the esteemed National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov), among them, *Codonopsis lanceolata* (NC_037949.1) and *Platycodon grandiflorus* (NC_035958.1) belongs to the Campanulaceae family, while the remaining plants belong to the Asteraceae family. The shared genes of mitogenomes were extracted using PhyloSuite[26] and aligned using MAFFT (v.7.505). Phylogenetic analysis was performed using the IQ-TREE (v 1.6.12)[36]. Bootstrap analysis with a value of 1000 was employed. Phylogenetic analysis results were portrayed using ITOL v6[37].

Collinear analysis

A comprehensive synteny diagram showing the relationship between *C. indicum* and closely related species was generated using the MCscanX [38]. Individual mitogenomes were compared two-by-two based on BLASTn (v 2.13.0)[24]. Conserved collinear blocks were identified and retained if homologous sequences were longer than 500 bp.

Results

Genomic features of two *C. indicum* mitogenomes

The complete mitochondrial genomes of two *C. indicum* were successfully assembled, with GC content of 45.50%. The genome sizes were 208,791 bp and 208,787 bp, respectively. Sequence comparison between the two *C. indicum* revealed a deletion of one base at position 9,987, an insertion of five bases between positions 179,090 and 179,091, a deletion of one base at position 206,051, and an insertion of

one base between positions 206,299 and 206,300 in white-rayed *C. indicum*. In the mitogenome assembly sketches of two *C. indicum*, three sequences were labeled as contig 1, contig 2, and contig 3. These sequences collectively represent the complete mitogenome of *C. indicum* (Figure S1 and S2).

Annotation of the two *C. indicum* mitogenomes identified 32 unique PCGs (24 core and eight non-core), 17 tRNA genes (three present in multiple copies), and three rRNA genes. These core PCGs sets comprised five ATP synthase genes (*atp1*, *atp4*, *atp6*, *atp8*, and *atp9*), nine Nicotinamide adenine dinucleotide dehydrogenase (NADH) genes (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, and *nad9*), four cytochrome C biogenesis genes (*ccmB*, *ccmC*, *ccmFC*, and *ccmFN*), three cytochrome C oxidase genes (*cox1*, *cox2*, and *cox3*), one membrane transport protein gene (*mttB*), one maturase gene (*matR*), one ubiquinol-cytochrome C reductase gene (*UQCRB*). The eight non-core PCGs included two ribosomal large subunit genes (*rpl5*, and *rpl10*), five ribosomal small subunit genes (*rps3*, *rps4*, *rps12*, *rps13*, and *rps19*), and one succinate dehydrogenase gene (*sdh4*) (Table 1 and Fig. 1).

Table 1
The Genetic makeup of mitogenomes in two *C. indicum*.

Group of genes	**Name of genes**
ATP synthase	*atp1*, *atp4*, *atp6*, *atp8*, *atp9*
NADH dehydrogenase	*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, *nad9*
Cytochrome b	*cob*
Cytochrome c biogenesis	*CcmB*, *ccmC*, *ccmFC*, *ccmFN*
Cytochrome c oxidase	*cox1*, *cox2*, *cox3*
Maturases	*matR*
Protein transport subunit	*mttB*
Ribosomal protein large subunit	*rpl5*, *rpl10*
Ribosomal protein small subunit	*rps3*, *rps4*, *rps12*, *rps13*, *rps19*
Succinate dehydrogenase	*sdh4*
Ribosome RNA	*rrn5*, *rrn18*, *rrn26*
Transfer RNA	*trnC-GCA*, *trnD-GUC*, *trnE-UUC*, *trnF-GAA*, *trnfM-CAU*, *trnG-GCC*, *trnH-GUG*, *trnI-CAU*, *trnK-UUU*, *trnM-CAU*, *trnN-GUU* (×2), *trnP-UGG*, *trnQ-UUG* (×2), *trnS-GCU* (×2), *trnT-GGU*, *trnW-CCA*, *trnY-GUA*
Codon usage analysis of PCGs	

Codon-usage analysis was performed on 32 unique PCGs found in the mitogenomes of two *C. indicum*. Notably, RSCU values were virtually identical between them (Fig. 2). With the exception of the initiation codon (AUG) and tryptophan (UGG), codon usage in the two *C. indicum* mitogenomes demonstrated preferences, as RSCU ≥ 1. For instance, alanine (Ala) exemplifies this trend by displaying a strong preference for GCU, marked by the highest RSCU value of 1.63 among the mitochondrial PCGs. Similarly, the termination codon exhibited a usage preference for UAA, with an RSCU value of 1.6. Additionally, the maximum RSCU of glutamine (Gln), histidine (His), and tyrosine (Tyr) values are greater than 1.5, and all had a strong codon usage bias (Table S1).

Repeat sequence analysis

Repetitive sequences are functionally versatile elements with profound impacts on genomic biology[39]. Firstly, they modulate genome stability by acting as recombination hotspots to facilitate gene rearrangement, inducing polymerase slippage mutations during DNA replication that cause insertions/deletions, and serve as target sites for transposon insertion, all of which disrupt gene structure, expression, and function[40]. Secondly, they participate in gene expression regulation, as a substantial proportion (7–10%) of transcription factor binding sites are derived from repetitive sequences, directly influencing transcriptional processes[41]. Thirdly, their interspecific divergence provides valuable markers for phylogenetic studies, enabling inferences of species relatedness[42]. Finally, they drive genome evolution through the "duplication-inactivation-neofunctionalization" paradigm, promoting the emergence of novel genes and biological functions, and contributing to adaptive diversification[43].

Repeat-sequence analysis of the two *C. indicum* mitogenomes uncovered 65 perfect microsatellites (SSRs). with monomeric and dimeric motifs accounting for 50.77% of the set (Figure. 3, Tables S2–S3). One hexameric SSRs was detected in the two *C. indicum* mitogenomes. 17 higher matching tandem repeat sequences were identified with a length between 16 and 61 bp (Table S4, and S5). Scattered repeat sequences were examined in the two *C. indicum* mitogenomes (Table S6, and S7). As shown in Fig. 3 The analysis of the two *C. indicum* mitogenomes revealed 149 pairs of repeat sequences, each with a length equal to or exceeding 30 bp. Among these repeats, 79 were palindromic in nature, while 69 were identified as forward repeats, with an additional 1 reverse repeat. Complementary repeats were not detected in either mitogenome. The longest palindromic repeat sequence and forward repeat sequence had lengths of 627 and 250 bp, respectively.

Migration of DNA from chloroplasts to mitochondria

Four homologous fragments originating from the chloroplast genome were identified within the two *C. indicum* mitogenomes (Fig. 4, Table S8, and S9). The homologous fragments detected in white-rayed *C. indicum* were 1,330 bp in length, constituting approximately 0.64% of the entire mitochondrial genome. This information was determined using the sequence similarity combined with the chloroplast genome. The mitochondrial genome of yellow-rayed *C. indicum* contains four homologous fragments, each measuring 1,325 bp in length. These fragments account for approximately 0.63% of the total

mitogenome. The lengths of the longest homologous segments in two *C. indicum* mitogenome were 1,087 bp and 1,082 bp, respectively. Additionally, seven complete genes were identified in the four homologous fragments, including two PCGs (*petG*, and *petL*), and five tRNA genes (*trnH-GUG*, *trnN-GUU*, *trnMCAU*, *trnP-UGG*, and *trnW-CCA*). In summary, the migration of DNA from chloroplasts to mitochondria in white-rayed *C. indicum* was highly similar to that in yellow-rayed *C. indicum*.

RNA editing in two *C. indicum* mitogenomes

Two *C. indicum* mitogenomes were analyzed, and within the 32 protein-coding genes of the mitogenome, revealed the presence of 501 potential RNA editing sites were identified. As shown in Fig. 5, among the mitochondrial protein-coding genes, *ccmB* stood out with the highest number of editing sites, amounting to 38 in total. Moreover, an impressive abundance of editing sites was observed within the *mttB* and *nad7* genes, surpassing the threshold of 30. The lowest number of editing sites in two *C. indicum* mitogenomes was atp6, with only one predicted RNA editing sites. In general, the number of RNA editing sites in white-rayed *C. indicum* mitochondrial PCGs was the same as that in the mitogenome of yellow-rayed *C. indicum*.

1. White-rayed *C. indicum*; (B) Yellow-rayed *C. indicum*.

Phylogenetic and Collinear analysis

A phylogenetic tree was constructed to explore the evolutionary connections among 37 species. This analysis relied on the DNA sequences of the 26 conserved mitochondrial PCGs (Table S9). Among them, *C. lanceolata* and *P. grandiflorus* were set as outgroups and belong to the Campanulaceae family. Among 37 species, the shared PCGs were *atp4*, *atp6*, *atp8*, *atp9*, *ccmB*, *ccmC*, *ccmFC*, *ccmFN*, *cox1*, *cox2*, *cox3*, *matR*, *mttB*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, *nad9*, *rpl16*, *rps3*, *rps12*, and *rps13*. The resulting phylogenetic tree showed strong support for most nodes. Specifically, 23 out of the 34 nodes had bootstrap support values exceeding 80%, while 7 nodes exhibited 100% support. The constructed phylogenetic tree supports two *C. indicum* belonging to Asterales and Asteraceae families. Two *C. indicum* formed a cohesive cluster alongside other *Chrysanthemum* species, aligning harmoniously with the contemporary categorization proposed by the Angiosperm Phylogeny Group (Group, 2016) (Fig. 6).

Synteny plots showed many homologous collinear blocks between *C. indicum* and closely related *Chrysanthemum* species. Notably, the mitochondrial genome sequences of two *C. indicum* exhibited high sequence homology, with only a few genomic rearrangements (Fig. 7). However, it is important to highlight that the colinear blocks between these nine mitogenomes within the *Chrysanthemum* genus were not aligned in the same order. These genomes undergo genomic recombination and exhibit a nonconservative arrangement.

Discussion

The high-quality genome of *C. indicum* enhances our understanding of the genetic basis underlying its ornamental and medicinal traits[44], providing valuable resources for germplasm conservation, pathway analysis, and breeding[45]. A previous study assembled a 3.11 Gb genome of *C. indicum*[46]. The organellar genomes of diploid and tetraploid *C. indicum* and their related species were assembled by Liu et al., they identifying structural variations and novel mitochondrial ORFs[18]. In Asteraceae, mitogenome sizes vary significantly, typically ranging from 200 to 400 kb. For instance, *Potentilla diversifolia* has a 360,751 bp mitogenome[47], while the *C. boreale* mitogenome is 211,002 bp[48]. Here, we assembled two *C. indicum* mitogenomes (208,791 and 208,787 bp), consistent with previously reported sizes. Their GC content (45.50%) aligns with *Bupleurum chinense*[18]. We identified 149 repetitive sequence pairs, that contribute to mitogenome stability[49]. Four homologous segments between the mitochondrial and chloroplast genomes indicate intracellular gene transfer (IGT), with variations observed in two *C. indicum*, which has been confirmed in previous studies[18]. Phylogenetic analysis clustered the two *C. indicum* with previously sequenced *C. indicum* (2x) and (4x), confirming their taxonomic placement within *Chrysanthemum*.

Codon usage preferences play a pivotal role in the gene expression process, influencing protein translation and the formation of the protein structure[50]. Current research on codon usage preferences primarily focuses on molecular evolution and translational regulation[51]. The number of tRNAs affected the frequency of codon usage and protein expression. Natural selection, gene expression, and protein structure effect codon usage bias[52]. Therefore, analyzing codon usage preferences can provide strategies for optimizing codon preferences, laying a theoretical foundation for utilizing genetic engineering technologies to improve plants[53]. In this study, the two *C. indicum* mitogenomes exhibited clear codon usage bias among their PCGs. Consistent with reports in other higher plants, the alanine codon family was strongly skewed toward GCU, which displayed the highest RSCU value in the mitogenome. Across all mitochondrial PCGs, A- or U-ending codons predominated, reflecting a marked bias toward A/U at the third codon position.

Plant mitochondria are one of the major sites for RNA editing and often occurr in mRNA[54]. RNA editing refers to the phenomena of base insertions, deletions, or conversions that occur in the coding regions of transcribed RNA. Nucleotide substitution is the most common mechanism for RNA editing. A previous study subjected comparative analyses of *Salvia miltiorrhiza*, *Arabidopsis thaliana*, and *Oryza sativa* revealed that a total of 1123 editing sites, with 225 sites involved C-to-U changes in the protein-coding regions of *S. miltiorrhiza*[55]. In Brassica napus, a total of 427 C-to-U sites were identified. In Arabidopsis, 441 C-to-U sites were identified[56]. It is worth noting that *Brassica napus* and Arabidopsis shared only 358 editing sites, accounting for 83% of the total editing sites[57]. In this study, RNA editing sites were predicted for two *C. indicum* mitogenomes. The mitochondrial PCG of both *C. indicum* genomes contained 501 potential RNA editing sites. All were edited from base C-to-U. These results are consistent with the C-to-U editing of RNA observed in most land plants. These findings contribute to a better understanding of the diversity and evolution of these plant groups, facilitating further research on their genetic variation, phylogeny, and systematics.

Conclusions

We assembled the mitogenome of two *C. indicum*. The complete mitogenomes of white-rayed and yellow-rayed *C. indicum* were 208,791 bp and 208,787 bp in genome size, respectively. 32 PCGs were annotated in the mitogenomes of two *C. indicum*. Notably, we observed codon usage bias in the mitochondrial PCGs of the two *C. indicum*, except for the initiation codon AUG and the codon for tryptophan (UGG). Furthermore, phylogenetic analysis of 26 common PCGs from 35 Asteraceae plants and two outgroup taxa strongly supported the placement of the two *C. indicum* species as sister taxa to *C. boreale*, in agreement with the latest classification proposed by the Angiosperm Phylogeny Group[58]. Notably, the mitochondrial genome sequences of two *C. indicum* exhibit high sequence homology, with only a few genomic rearrangements occurring. These findings emphasize the conservation and stability of mitogenomes within the *C. indicum*. In summary, the characterization of two *C. indicum* mitogenomes will provide valuable insights for evolutionary and phylogenetic studies of *Chrysanthemum* and Asteraceae.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors explicitly state that the research was conducted devoid of any commercial or financial affiliations that may create a perception of potential conflicts of interest.

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Author Contribution

The manuscript was conceptualized and designed by YHT. YHT, LXF, SZH, and WC were responsible for data analysis and manuscript writing. WM, MLJ, and YHT provided experimental samples. WC and YHT conducted manuscript editing. Additionally, SC performed language editing and provided final approval

for the manuscript. All authors made significant contributions to the article and have given their approval for the submitted version.

Data Availability

The mitochondrial genome of *C. indicum* has been submitted to the NCBI database (https://www.ncbi.nlm.nih.gov/) under the accession number PP350832 (white-rayed *C. indicum*) and PP350833 (yellow-rayed *C. indicum*).

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Figures

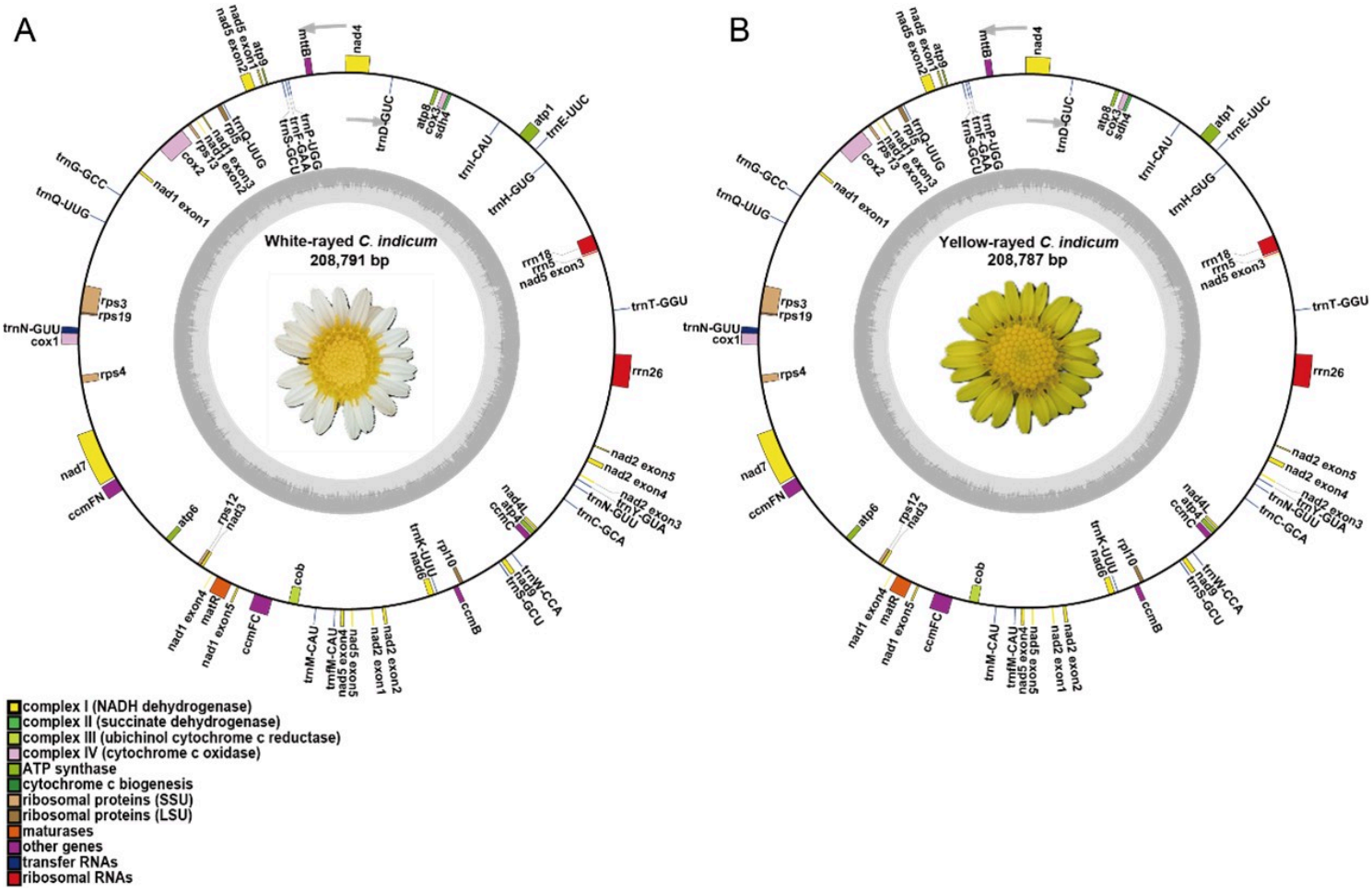

Figure 1

Mitogenomes structure and annotation of two *C. indicum*. (A) White-rayed *C. indicum*; (B) Yellow-rayed *C. indicum*.

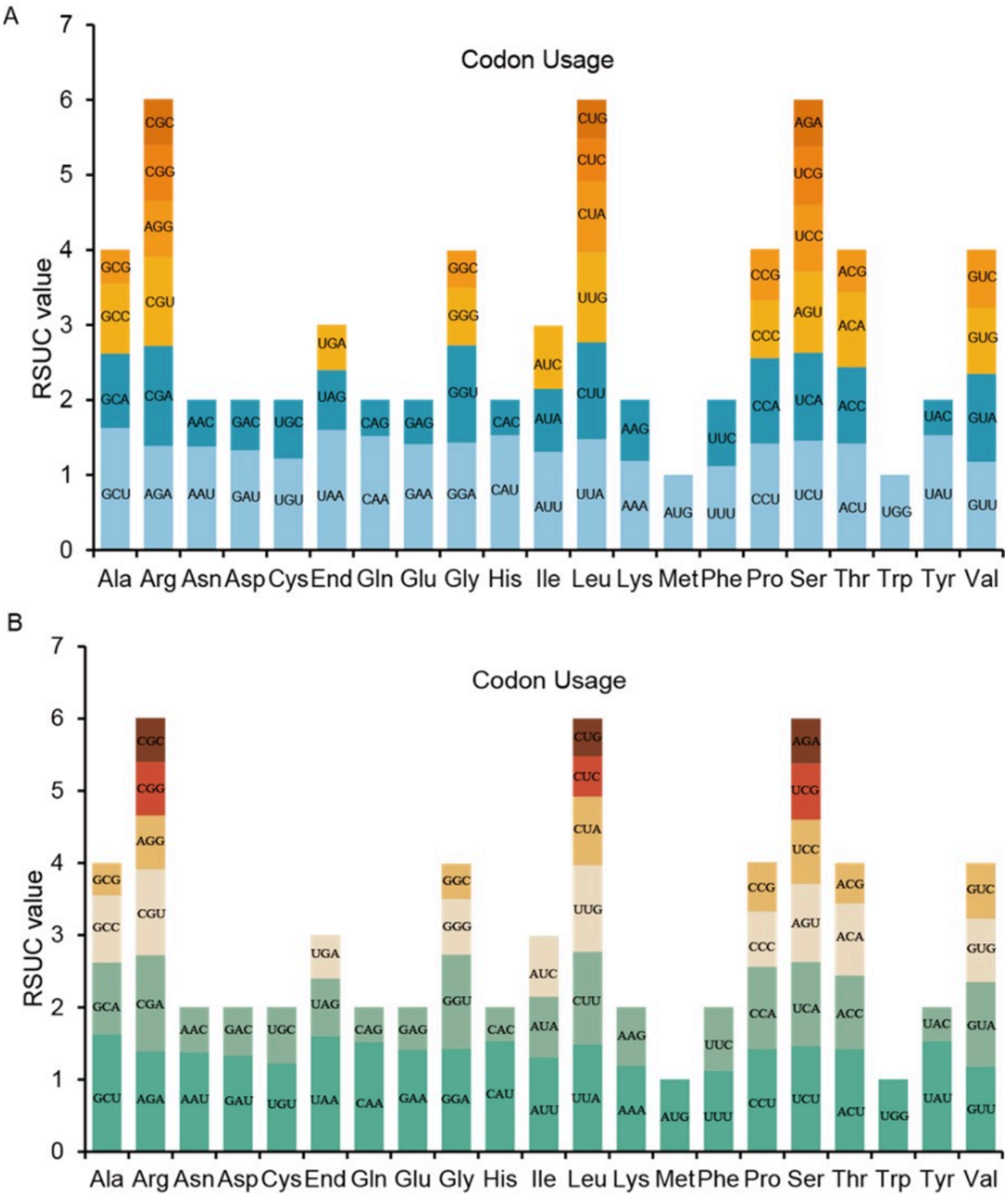

Figure 2

Relative synonymous codon usage of two *C. indicum* mitogenome PCGs. (A) White-rayed *C. indicum*; (B) Yellow-rayed *C. indicum*.

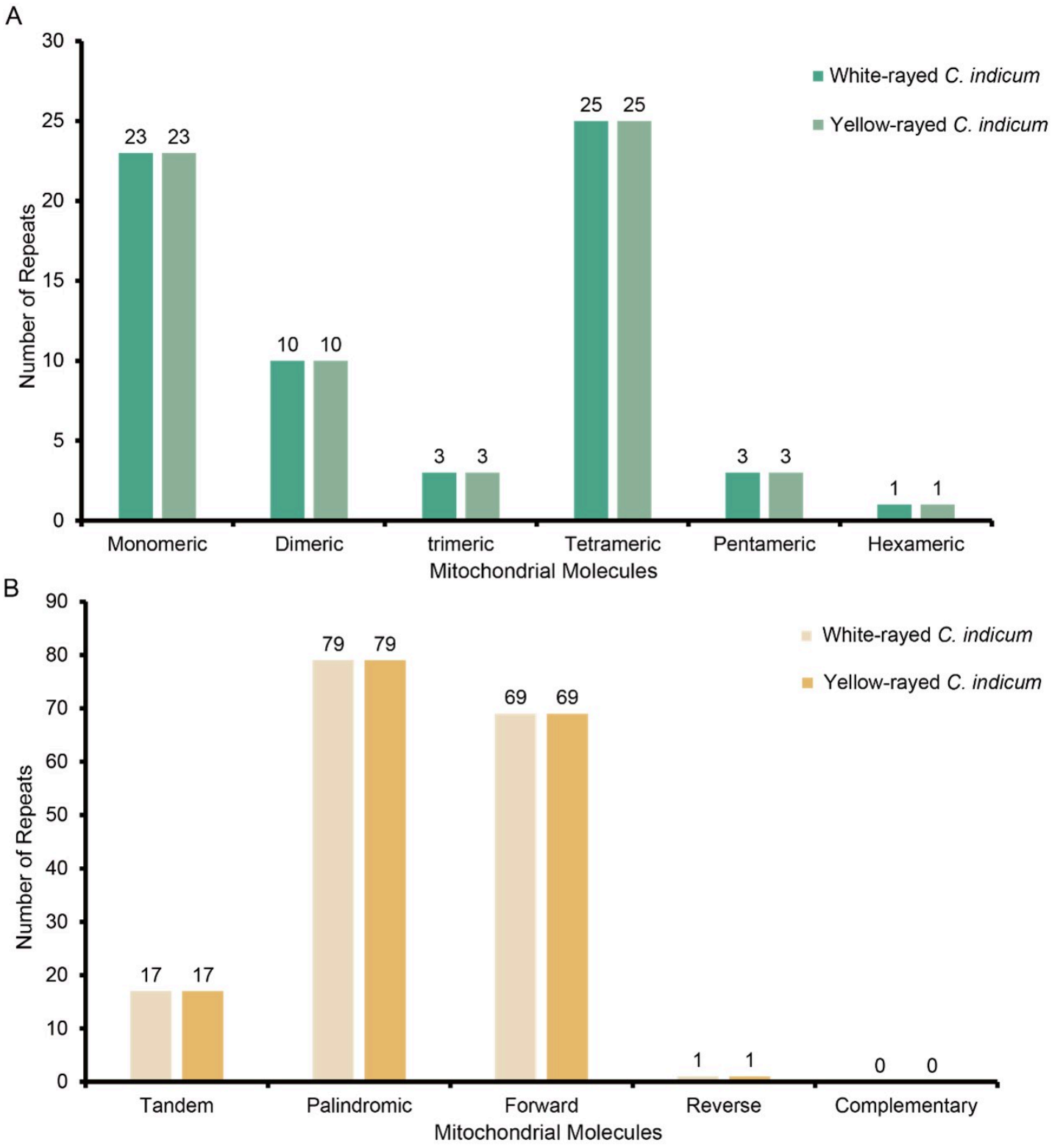

Figure 3

Number of repetitive sequences in two *C. indicum* mitochondrial genomes. (A) White-rayed *C. indicum*; (B) Yellow-rayed *C. indicum*

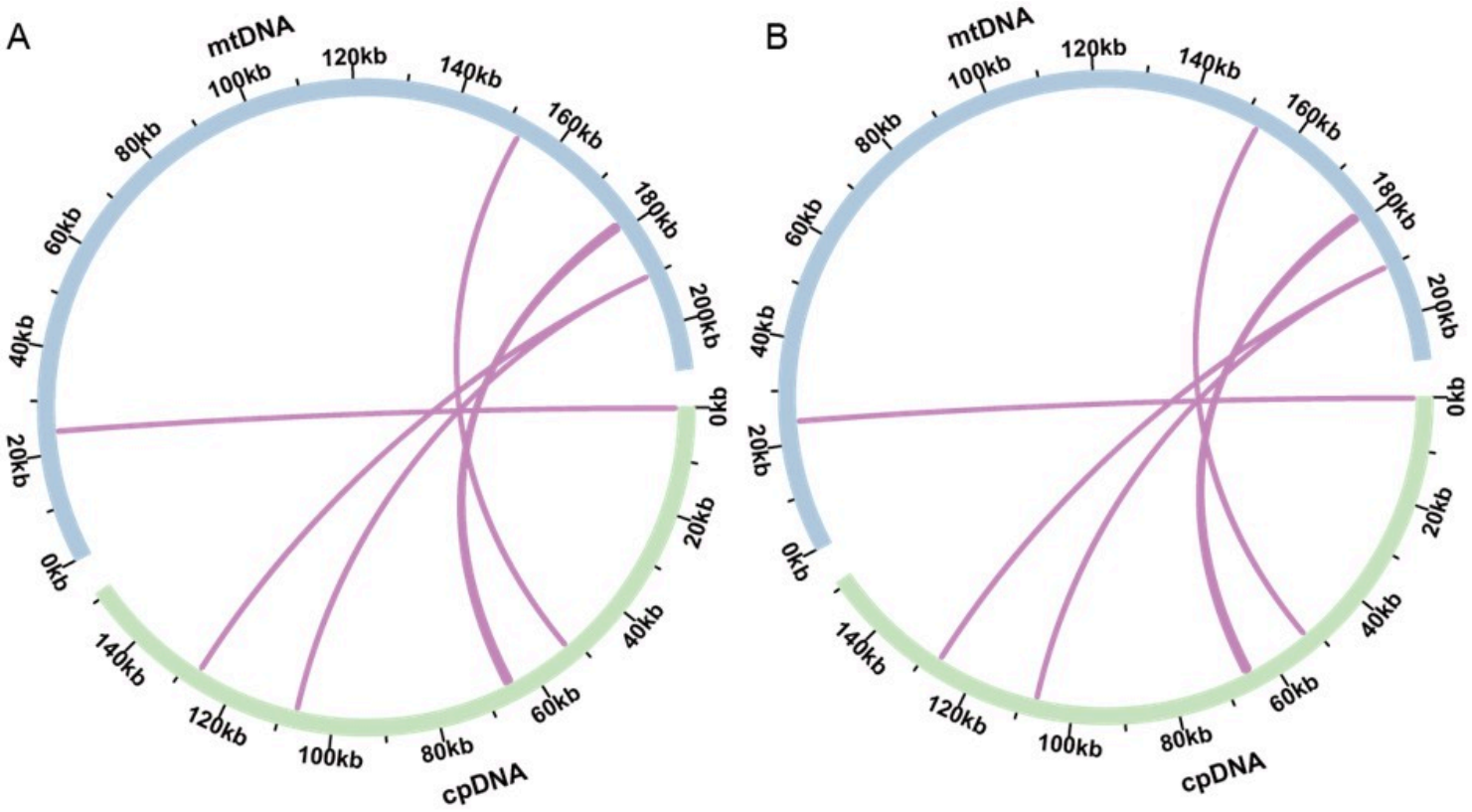

Figure 4

Homologous sequences of mitochondria and chloroplasts in two *C. indicum*. Blue arcs: mitochondrial genome, green arcs: chloroplast genome. The pink lines: homologous genomic fragments. (A) White-rayed *C. indicum*; (B) Yellow-rayed *C. indicum*.

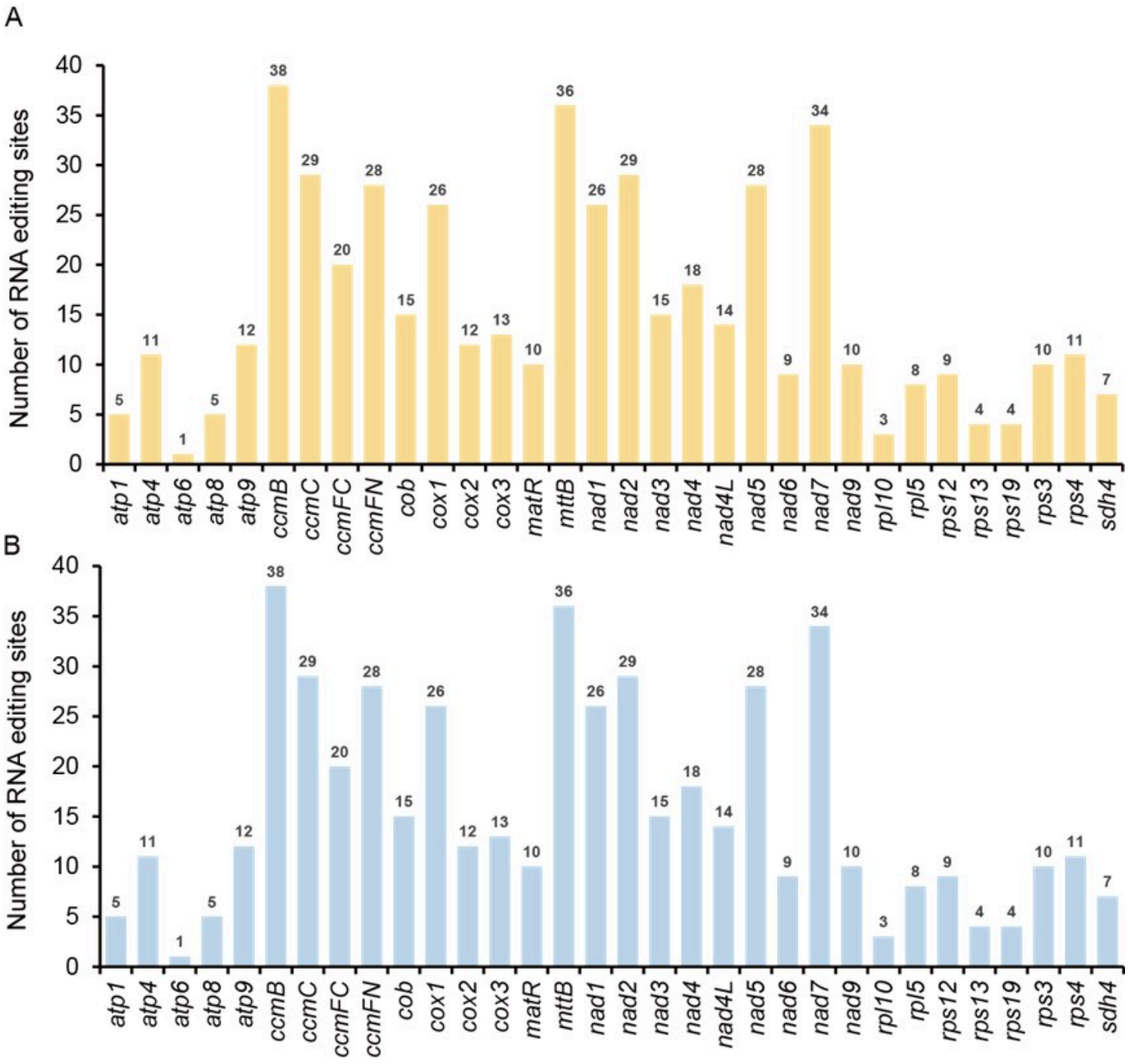

Figure 5

RNA editing events of different genes in two *C. indicum* mitogenomes.

(A) White-rayed *C. indicum*; (B) Yellow-rayed *C. indicum*.

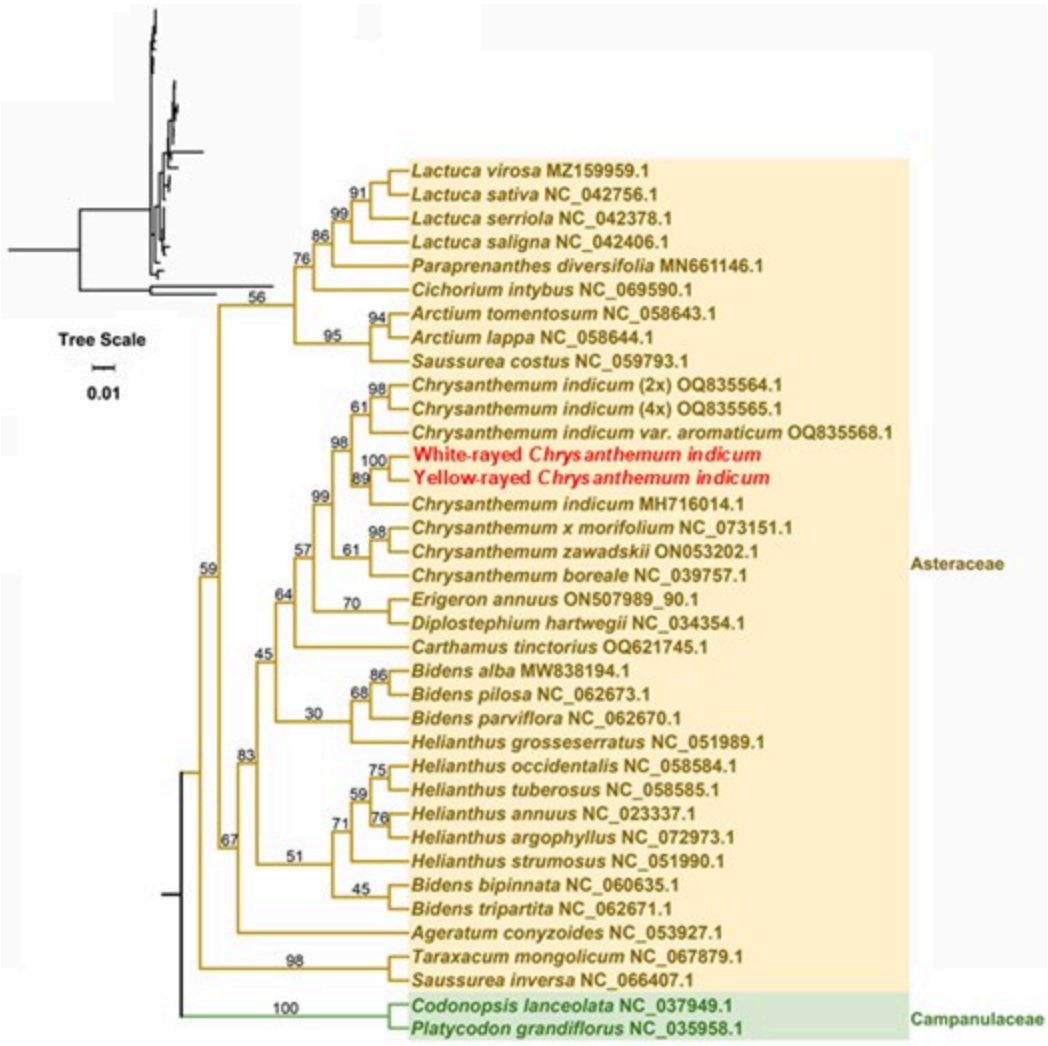

Figure 6

Phylogenetic relationships of two *C. indicum* with other 35 plant species.

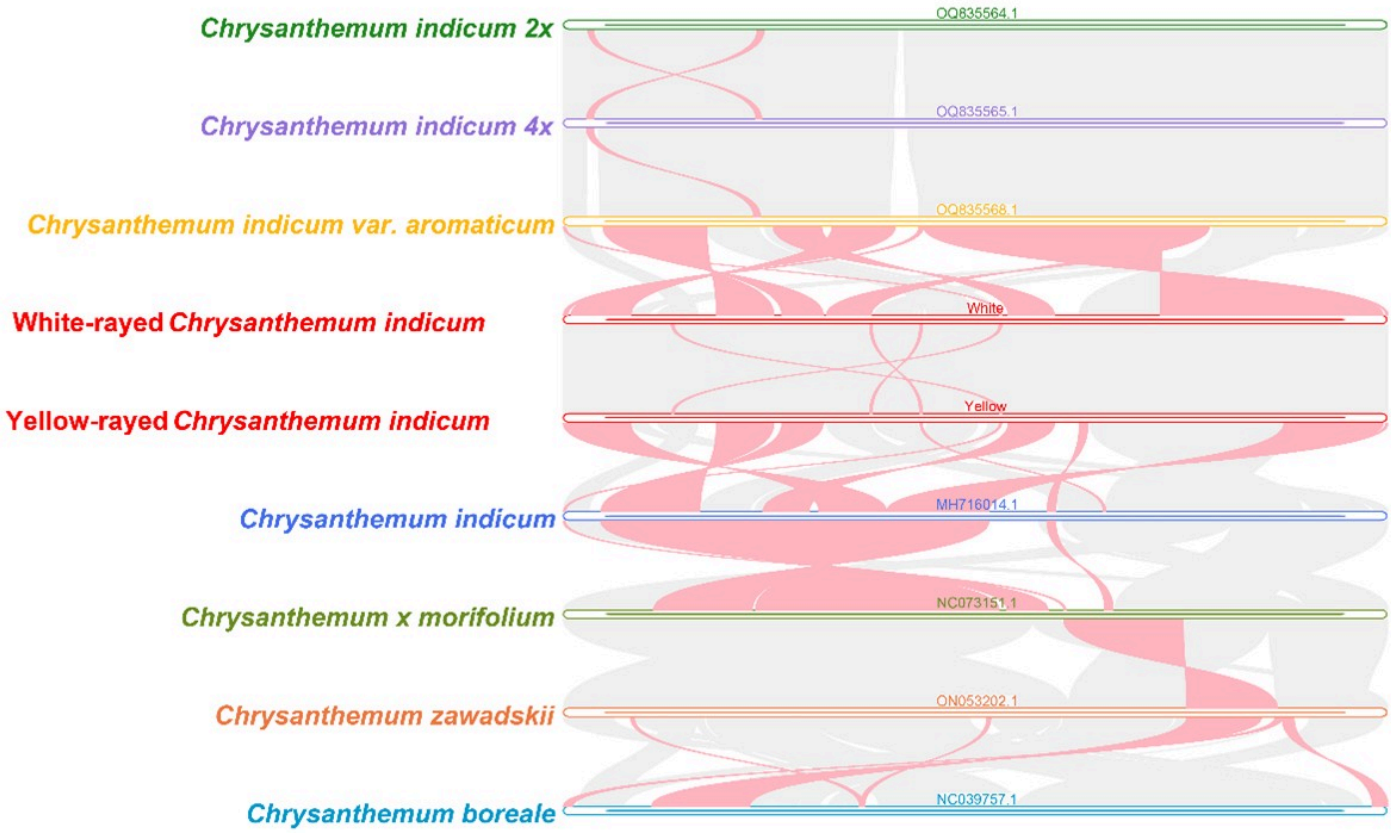

Figure 7

Collinearity analysis of the nine *Chrysanthemum*plants.

Supplementary Files

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