Comparative analysis of the mitochondrial and chloroplast genomes of Purple nutsedge (Cyperus rotundus), a noxious weed

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Research Article

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Abstract

Background *Cyperus rotundus* L. is a notorious weed that harms agricultural ecosystems worldwide. Although the chloroplast genome (cpgenome) of *C. rotundus* has been studied, there have been no reports on the mitochondrial genome (mitogenome) of *C. rotundus*.

Results The mitogenome and the cpgenome of *C. rotundus* XFZ01 have four circular DNA moleculesand one circular DNA molecule, respectively. Their total lengths were 1,491,358 bp and 186,119 bp, respectively. The mitogenome contained 75 genes, including 40 protein-coding genes (PCGs), 9 rRNA genes, and 26 tRNA genes. The cpgenome contained 121 genes, including 69 PCGs, 8 rRNA genes, and 44 tRNA genes. Analysis of repetitive sequences identified 350 and 88 SSRs, 144 and 123 tandem repeats, 686 and 1,210 interspersed repeats in the mitogenome and cpgenome, respectively. Homologous fragment analysisindicated that 11 homologous fragments migrated from the cpgenome to the mitogenome. Codon preference analysis showed that both the mitogenome and cpgenome had weak codon preferences. Furthermore, cpgenome PCGs had 23 RNA editing sites compared to 13 in mitogenome PCGs. Phylogenetic analysis verified that *C. rotundus* had the closest genetic relationship with *C. esculentus.* Finally, Ka/Ks research showed that most mitogenomic PCGs, except for the *nad6* gene, undergo negative selection.

Conclusions In this study, we assembled and annotated the mitogenome of the noxious weed *C. rotundus* and conducted a differential analysis using its cpgenome. These results lay a theoretical foundation for understanding the genetic variation, phylogeny, and population control of *C. rotundus*.

Background

Cyperus rotundus L., also called purple nutsedge, belongs to the *Cyperus* genus in the Cyperaceae family. It mainly occurs in the dryland fields of tropical, subtropical, and temperate regions [1]. *C. rotundus* has a rapid reproductive ability and an extensive underground tuber system, which seriously affects the production of crops such as sugarcane, corn, and soybeans [2, 3]. It is regarded as one of the most pervasive, troublesome, and financially detrimental agricultural weeds [4]. *C. rotundus* harms crops in three ways: it competes with other crops for sunlight, water, nutrition, and living space, resulting in reduced crop yields [5, 6]. Furthermore, *C. rotundus* affects crop production through allelopathy, which inhibits seed germination and the growth and development of crops [7]. Finally, *C. rotundus* is the host and transmission vector of several pathogenic bacteria and pests, leading to further crop damage and promoting the occurrence and spread of pests and diseases [8]. Although herbicides may hinder the development of *C. rotundus*, they do not completely inhibit their regeneration potential or tuber vitality of *C. rotundus* [4]. Previous studies have shown that many herbicides, such as paraquat, atrazine, and neburon, target the molecular mechanisms in the mitochondria and chloroplasts by interfering with their normal operation [9–11]. Among these, the D1 protein of photosystem II, encoded by the chloroplast gene *psbA*, is an important target of commercial herbicides [12, 13]. Therefore, studying *C. rotundus* organellar genomes could provide a theoretical basis for improving control strategies.

Organellar genomes consist of mitochondrial genome (mitogenome) and chloroplast genome (cpgenome) [14, 15]. Most cpgenomes have a conventional four-segment structure consisting of a pair of inverted repeat (IR) sequences, a large single-copy (LSC) region, and a small single-copy (SSC) region [16, 17], and the IR region stabilizes its structure [18]. The mitogenome is longer and has a more complex structure than the cpgenome. The non-coding region of the mitogenome is composed of exogenous migratory fragments and recombination of their repetitive sequences, indicating poor evolutionary conservation of the sequences in this region [19]. However, the cpgenome has the advantages of single-parent inheritance, stable structure, coding genes, a moderate overall evolution rate, a slow evolution rate of coding genes, and a fast evolution rate of non-coding sequences. Therefore, it has become an important tool for studying plant phylogeny, species identification, intraspecific or interspecific cytoplasmic diversity, and genetic engineering [20–25]. The combination of organellar genome data provides new insights into species evolution [26, 27].

At present, there is no research on methods of eradication of *C. rotundus*, and there are few reports on weed organellar genomes, especially the mitogenome. Studies on the organellar genome of *C. rotundus* can lay the foundation for understanding the genetic variation, phylogeny, and population control. They also provide valuable information for studying weed organellar genomes. In this study, the organellar genomes of *C. rotundus* XFZ01 were assembled and annotated, and repeat sequence, gene transfer, codon preference, RNA editing prediction, phylogeny, and selective pressure were systematically analyzed.

Results

Annotation of C. rotundus organellar genomes

The *C. rotundus* mitogenome has four circular DNA molecules, namely, mt1 (mitochondrial chromosome 1), mt2, mt3, and mt4, with mt1 being the main circular DNA molecule. The lengths of mt1, mt2, mt3, and mt4 were 706,916, 399,727, 332,552, and 52,163 bp, respectively, and GC contents were 40.66%, 40.55%, 40.71%, and 41.02%, respectively (Table S1). In Fig. 1 and Table 1, the functional categories and physical locations of the annotated genes are displayed. There were 19 (including 14 core genes and 5 variable genes), 11 (including 9 core genes and 2 variable genes), and 10 (including 8 core genes and 2 variable genes) protein-coding genes (PCGs) in mt1, mt2, and mt3, respectively. mt1, mt2, and mt3 had three, four, and one rRNA, respectively. There were nine, ten, and seven tRNAs in mt1, mt2, and mt3, respectively. However, mt4 contains only one rRNA gene (*rrn18*).

Table 1
Gene annotation information of *C. rotundus* mitogenome

Group of genes		Name of genes			
		mt1	**mt2**	**mt3**	**mt4**
Core genes	ATP synthase	*atp6*	*atp1*, *atp8*, *atp9*	*atp4*, *atp8*	
	NADH dehydrogenase	*nad1*(x2), *nad2*, *nad4*, *nad5*(x2), *nad6*, *nad9*	*nad1*, *nad2*, *nad3*, *nad7*	*nad1*, *nad2*, *nad4L*, *nad5*	
	Cytochrome c biogenesis	*cob*			
	Ubiquinol cytochrome c reductase	*ccmFC*, *ccmFN*	*ccmC*	*ccmB*, *ccmC*	
	Maturase	*matR*			
	Transport membrane protein	*mttB*			
	Cytochrome c oxidase	*cox2*, *cox3*	*cox1*		
Variable genes	Ribosome large subunit	*rpl5*		*rpl16*	
	Ribosome small subunit	*rps1*, *rps4*, *rps7*, *rps13*	*rps4*, *rps12*	*rps3*	
rRNA genes	Ribosome RNA	*rrn18*(x2), *rrn26*	*rrn5*(x3), *rrn26*	*rrn26*	*rrn18*
tRNA genes	Transfer RNA	*trnE-TTC*, *trnK-TTT*, *trnM-CAT* (x3), *trnR-GCG*, *trnS-GCT*, *trnS-TGA*, *trnY-GTA*	*trnC-GCA*, *trnD-GTC* (x2), *trnE-TTC*, *trnH-GTG*, *trnM-CAT*, *trnN-GTT*, *trnQ-TTG*, *trnS-GGA*, *trnW-CCA*	*trnC-GCA*, *trnD-GTC*, *trnF-GAA*, *trnM-CAT*, *trnP-TGG*, *trnQ-TTG*, *trnS-GCT*	

Note: Bracketed numbers indicate the number of gene repeats.

The length of *C. rotundus* cpgenome was 186,119 bp, consisting of two reverse repeat (IR, 74,843 bp) sequences, a short single copy (SSC, 10,315 bp) sequence, and a long single copy (LSC, 100,961 bp) sequence (Fig. S1). *C. rotundus* genome had a GC content of 33.19% (Table S1). Specifically, the LSC, SSC, and IR regions had GC contents of 29.85%, 18.6%, and 46.5%, respectively. The cpgenome was annotated with 121 genes, including 69 PCGs, 8 rRNA genes, and 44 tRNA genes (Table S2).

Repetitive sequences of C. rotundus organellar genomes

Microsatellite DNA, also known as Simple Sequence Repeats (SSRs), is a tandem repeat sequence consisting of several nucleotides (usually 1–6) as repeating units with lengths of 50–100 bp. It is commonly found in eukaryotic genomes [28]. The mitogenome and cpgenome of *C. rotundus* included 350 and 88 SSRs, respectively (Fig. 2A-B, Table S3, and S4). In the mitogenome, monomers and dimers accounted for 55.14% of all SSRs. Among the 97 monomer SSRs, adenine (A) and thymine (T) monomers accounted for 44.33% and 55.67%, respectively. AT repeats were the most frequent dimeric SSRs, accounting for 55.21% of all dimeric SSRs. The most common SSRs were tetranucleotides, accounting for 30.57% of all the SSRs. However, monomer SSRs were the most abundant in the cpgenome, accounting for 64.77% of the total SSRs. Among the 57 monomer SSRs, A and T monomers accounted for 45.61% and 54.39%, respectively. Furthermore, these SSRs can potentially serve as *C. rotundus* identification markers.

Tandem repeats consist of multiple copies of repeating units of ≥ 7 nucleotides located next to one another [29]. The mitogenome contained 144 tandem repeats, with lengths ranging from 2 to 70 bp (Fig. 2A, Fig. 2C, and Table S5). There were 123 tandem repeats in the cpgenome, with lengths ranging from 2 to 73 bp (Fig. 2A, Fig. 2C, and Table S6). These repeats will be further evaluated in future studies for their potential as molecular markers.

Interspersed repeats are crucial for genetic diversity and assist in plant genome evolution [30]. In total, 686 interspersed repeats were found in the mitogenome (Table S7), including 312 palindromic repeats, 371 forward repeats, 1 reverse repeat, and 2 complementary repeats (Fig. 2A and Fig. 2C). Simultaneously, 1,210 interspersed repeats were identified in the cpgenome (Table S8), including 394 palindromic, 797 forward, 10 reverse, and 9 complementary repeats (Fig. 2A and Fig. 2C). The proportion of forward repeats in both the mitogenome and cpgenome was the highest at 54.08% and 65.87%, respectively.

Homologous fragments analysis of C. rotundus organellar genomes

In the *C. rotundus* annotated mitogenome and the cpgenome, 11 identical fragments were found in both genomes (Fig. 3 and Table S9). The 11 fragments comprised 3,901 bp in total, accounting for 0.26% of the mitogenome and 2.10% of the cpgenome. The longest aligned fragment was 741 bp, and the shortest aligned fragment was only 60 bp. The cp DNA migrated six fragments to mt1 DNA, containing one complete gene (*trnM-CAU*) and four partial genes (*rrn16*, *rrn18*, *rrn26*, and *petA*). The cp DNA migrated two fragments to the mt2 DNA, containing four partial genes (*rrn5*, *rrn16*, *rps15*, and *ndhH*). The cp DNA

migrated as two fragments to mt3 DNA, containing one complete gene (*trnM-CAU*) and one partial gene (*rpl16*). The cp DNA migrated from one fragment to mt4 DNA, containing two partial genes (*rrn16* and *rrn18*). These fragments included one complete gene (*trnM-CAU*) and eight partial genes (*rrn16*, *rrn18*, *rrn5*, *rrn26*, *rps15*, *rpl16*, *petA*, and *ndhH*). Among these, the sequence fragments of eight partial genes underwent a certain degree of loss, indicating that these genes may be non-functional.

- **Codon preference of C. rotundus organellar genomes**

The codon makeup of the organellar genome of *C. rotundus* was examined using a self-coding Perl script. In the mitogenome, the codon numbers of mt1, mt2, mt3, and mt4 in all PCGs were 13,492, 7,560, 6,352, and 1,004, respectively (Table 2). The average GC content across all GC, as well as the GC1, GC2, and GC3 contents, was less than 41.50%. The effective number of codons (ENC) was higher than 56.24, indicating that the mitogenome has a weak codon preference. There were 30 codons with a relative synonymous codon usage (RSCU) > 1 (Fig. 4), which was the same across all four chromosomes, demonstrating that the usage of these codons was higher than that of other synonymous codons. The *C. rotundus* cpgenome had 3,761 codons, and its average GC content, as well as the contents of GC1, GC2, and GC3, were all less than 33.70% (Table 2). It also exhibited a weak codon preference based on its ENC, which was 52.86. Additionally, there were 29, 2, and 33 codons with RSCU values > 1, = 1, and < 1, in the *C. rotundus* cpgenome, respectively (Fig. 4). Similar to the mitogenome, the RSCU value of AGA was the highest among the codons used, exceeding 2.10, indicating that this codon was the most frequently used in the *C. rotundius* organellar genome.

Table 2
Overall features of codon usage in the *C. rotundus* organellar genomes

Genome	Codon number	GC1	GC2	GC3	GC all	ENC
mt1	13,492	40.49	40.91	40.60	40.67	56.260
mt2	7,560	40.66	40.44	40.54	40.55	56.324
mt3	6,352	40.31	40.99	40.81	40.70	56.237
mt4	1,004	41.41	40.40	41.23	41.01	56.439
cp	3,761	33.62	32.54	33.43	33.19	52.860

- **Prediction of RNA editing of C. rotundus organellar genomes**

RNA editing, which refers to the addition, loss, or conversion of bases in the coding region of transcribed RNA, is a common phenomenon in organellar genomes [31]. In this study, the RNA-editing events in *C. rotundus* organellar genomes had been focused. The number of PCGs that underwent RNA editing events was determined to be nine for the mitogenome (Fig. 5A) and nine for the cpgenome (Fig. 5B). There were 13 and 23 RNA editing events in the mitogenome (Table S10) and the cpgenome (Table S11), respectively. In the PCGs of the mitogenome, most RNA-editing sites were found in *nad1*, followed by *atp1* and *ccmB* with 3, 2, and 2 sites, respectively. In the PCGs of the cpgenome, most RNA editing sites were

discovered in *ndhB*, followed by *rpoC2* and *ndhC*, with 7, 5, and 3, respectively. The mitogenome contained five different RNA editing types, whereas the cpgenome contained six different RNA editing types (Fig. 5C). The C-to-T editing type predominated in both the mitogenome and the cpgenome. In terms of RNA editing efficiency, *C. rotundu*s had 7 (53.85%) mitochondrial PCGs and 17 (73.91%) chloroplast PCGs with editing efficiencies greater than 80% (Fig. 5D). After RNA editing, the *C. rotundu*s mitogenome retained 15.38% of the hydrophilic amino acids and 61.54% of the hydrophobic amino acids, while changing 23.08% of the hydrophilic amino acids into hydrophobic amino acids. In the *C. rotundu*s cpgenome, 26.09% of the hydrophobic amino acids and 13.04% of the hydrophilic amino acids were unaltered, 4.35% of the hydrophobic amino acids were converted to hydrophilic amino acids, and 56.52% of the hydrophilic amino acids were converted to hydrophobic amino acids .

- **Phylogenetic analysis of C. rotundus organellar genomes**

To identify the phylogenetic relationship of *C. rotundus* organellar genome, a phylogenetic tree was constructed using 17 mitochondrial and 64 chloroplast PCGs shared by 10 related plants, with *Arabidopsis thaliana* and *Brassica napus* as the outer group. The 17 mitochondrial genes in all 10 plants were *nad1*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *nad7*, *cob*, *cox1*, *cox3*, *atp1*, *atp6*, *atp9*, *rps3*, *rps4*, *rps12* and *ccmC*. The chloroplast genes used to construct the phylogenetic tree were 64 chloroplast PCGs of *C. rotundus*, in addition to six genes, *atpE*, *psaJ*, *rpl 22*, *rps16*, *ycf3* and *ycf4*, as they were more or less missing in the cpgenomes of 10 plant species. Phylogenetic trees constructed based on mitochondrial and chloroplast genes were identical, and the results showed that the phylogenetic relationship between *C. rotundus* and *C. esculentus* was the closest (Fig. 6).

- **Evolutionary selection pressure analysis of C. rotundus mitogenome**

Seventeen PCGs of *C. rotundus* mitogenome were calculated using the non-synonymous substitution rate (Ka) and synonymous substitution rate (Ks). As shown in Fig. 7A, the Ka/Ks value of *nad6* was > 1, indicating that it had undergone positive selection. In contrast, the Ka/Ks values of the other genes were < 1, indicating negative selection. In particular, *atp9*, *cox1*, *nad4L*, and *rps12* had low Ka/Ks values with the smallest variations, demonstrating that they are crucial to the function of the mitogenome. Figure 7B shows the pairwise Ka/Ks values for the ten different species. In addition to the Ka/Ks values of *C. rotundus nad6* gene, the differences in the Ka/Ks values of these species were not particularly noteworthy.

Discussion

Compared with the cpgenome reported by Wu et al. [32], the cpgenome of *C. rotundus* XFZ01 increased by 3,133 bp in length, with an increase of three PCGs (*rps8*, *rpl14*, and *rpl16*) and three tRNAs (*trnI-CAU*, *trnA-GUC*, and *trnStop-UUA*). however, two PCGs (*rps3* and *atpF*) and two tRNAs (*trnD-GUC* and *trnL-UAA*) were absent. *C. esculentus* organellar genomes have been studied, and the mitogenome is the only one that has been assembled and annotated in studies of mitogenomes in Cyperaceae [33, 34]. The mitogenome of *C. rotundus* has four circular DNA molecules, whereas *C. esculentus* has only one circular

DNA molecule, indicating that the structure of the *C. rotundus* mitogenome is more complex. Previous studies have shown that GC content can affect species ecology, distribution, environmental adaptation, and life history [35]. The overall GC contents of *C. roundus* mitogenome and *C. esculentus* mitogenome were approximately 40.65% and 40.77%, respectively. Their cpgenomes had the same overall GC content (33.19%).

Important genetic markers, such as repetitive sequences, are naturally polymorphic and are often used to study intraspecific genetic variations and diversity in plants [36]. In total, 438 SSRs were obtained from the *C. rotundus* mitogenome and cpgenome. These results provide many reference markers for assessing *C. rotundus* germplasm diversity and species identification. Interspersed repeats have been shown to regulate gene expression and influence plant phenotypic characteristics [37]. In the present study, there were 686 interspersed repeats in the *C. rotundus* mitogenome and 1,210 interspersed repeats in the *C. rotundus* cpgenome. However, their effects on the phenotypic characteristics of *C. rotundus* require further investigation.

The discovery of mitochondrial plastid DNAs (MTPTs) helped in the correct assembly of organellar genomes and comprehension of the evolution of organellar genomes [38, 39]. Jang et al. found that the MTPT regions are mutational hotspots [40]. The results demonstrated that 11 segments, which were probably MTPT sequences, moved from the cpgenome to the mitogenome of *C. rotundus*. These segments contain one complete gene that may participate in the normal execution of the function and eight incomplete genes that may be non-functional.

Codon preference has two regulatory effects on gene expression: the first type directly regulates the expression of the gene itself that uses the codon [41], and the second type indirectly affects the expression of other genes in the genome through the competitive consumption of tRNA [42]. Gene expression levels were determined using ENC values. An ENC value closer to 20 indicated higher gene expression levels and stronger codon preference. Conversely, an ENC value closer to 61 indicates lower gene expression levels and weaker codon preferences [43, 44]. In the organellar genome analysis of *C. rotundus*, the ENC value of the cpgenome was 53.86, and that of the mitogenome was > 56.24. This indicated that the codon preference of the mitogenome was weaker than that of the cpgenome. Additionally, the RSCU value represents the proportion between the codon's actual and theoretical usage frequencies in the absence of usage bias. If the RSCU value is < 1, the codon is utilized less frequently; if RSCU value is = 1, the codon is applied in an unbiased manner; and if RSCU value is > 1, the codon is utilized more frequently [45, 46]. The results of this study indicate that the organellar genome of *C. rotundus* contains 59 codons with RSCU value of > 1, with most codons ending with A/U bases.

The higher plant organellar genomes undergo RNA editing as a post-transcriptional mechanism, which enhances protein folding [47, 48]. 13 and 23 RNA editing sites were found in 9 PCGs of the *C. rotundus* mitogenome and 9 PCGs of the *C. rotundus* cpgenome, respectively. Compared with the organellar genomes of *Punica granatum*, *Pereskia aculeata*, and *Quercus acutissima*, *C. rotundus* has fewer RNA editing sites [49–51]. Most RNA modifications occur at secondary codon positions [45, 52]. However, only

23.07% of RNA editing sites in the *C. rotundus* mitogenome were found at the second codon position. The discovery of RNA editing sites in the organellar genomes of *C. rotundus* can help elucidate the gene function prediction of novel codons. RNA editing occurs widely in plant organellars and is essential for plant growth and development [53]. However, the mechanisms underlying RNA editing remain unclear.

The organellar genome is very conserved and is commonly used to construct phylogenetic evolutionary trees to study the species classification and evolutionary status of plants [54–56]. The results of the phylogenetic analysis showed that *C. rotundus* and *C. esculentus* were relatively close, which is consistent with previous observations obtained through evolutionary analysis [33]. Moreover, Niemeyer et al. found that the morphological characteristics, growth habits, and developmental processes of *C. rotundus* and *C. esculentus* are very similar [57]. This result further supports the phylogenetic relationship between *C. rotundus* and *C. esculentus*.

Analysis of the 17 PCGs in the mitogenome of *C. rotundus* revealed that most PCG genes were neutral or negatively selected. Only the Ka/Ks value of the *nad6* gene, which was > 1 indicated that the gene may have undergone positive selection. Analysis of the pairwise Ka/Ks values between the species showed that only the *nad6* gene of *C. rotundus* always had Ka/Ks values > 1. According to the description of the *nad6* gene by Yamato et al., the gene is one of the plant mitogenome genes with the fastest rate of sequence evolution [58]. It can be speculated that this gene was crucial for the evolution of *C. rotundus*, but this requires further verification.

Conclusions

In this study, the *C. rotundus* mitogenome is reported for the first time. The mitogenome consists of four circular DNA molecules with a total length of 1,491,358 bp. The mitogenome contained 75 annotated genes, including 40 PCGs, 9 rRNA genes, and 26 tRNA genes. The cpgenome consisted of one circular DNA molecule, and was 186,119 bp in length. The cpgenome contained 121 annotated genes, including 69 PCGs, 8 rRNA genes, and 44 tRNA genes. The repeat sequences, gene transfer, codon preference, and RNA editing predictions of *C. rotundus* organellar genomes were analyzed. In addition, the phylogenetic analysis showed that *C. rotundus* had the closest genetic relationship with *C. esculentus*. Ka/Ks analysis demonstrated that most PCGs underwent negative selection, indicating that genes in the organellar genomes were conserved throughout evolution. This study offers a theoretical foundation for understanding the genetic variation, phylogeny, and population control of *C. rotundus.*

Methods

Plant materials, DNA extraction, and sequencing

C. rotundus was collected from Fusui, Guangxi, China (Longitude:107.79 E, Latitude: 22.52 N), and the plant specimen was named XFZ01. It was subsequently planted at the Agricultural College Planting Base of Guangxi University. Fresh leaves of *C. rotundus* were collected and immediately frozen in liquid

nitrogen. The total DNA of *C. rotundus* leaves was extracted using a DNA extraction kit from Nanjing Novozan Biotechnology Co., Ltd. (Nanjing, China). The quality and integrity of the total DNA of *C. rotundus* were detected using 1.5% agarose gel electrophoresis and a Nanodrop 2000. A SQK-LSK 109 connection kit was used to create a DNA library. To obtain a more complete and accurate mitochondrial and chloroplast genome of *C. rotundus*, the second-generation (Illumina, San Diego, CA, USA) and third-generation (Nanopore, Wilmington, DE, US) sequencing technologies were used to obtain a more complete and accurate mitochondrial and chloroplast genomes of *C. rotundus*.

Assembly and annotation of genome

A preliminary set of mitochondrial and chloroplast genome data was obtained using Miniasm software [59]. Filter and correct contigs were obtained using Porecop (https://github.com/rrwick/Porechop/) and Racon (https://github.com/isovic/racon.git racon/) software [60, 61]. The XFZ01 organellar genome was annotated using the mitogenome (NC058697) of *C. esculentus* and the cpgenome (MT937176) of *C. rotundus* as reference genomes. The assembly from scratch was performed using the Flye and Canu software [62, 63]. Minimap2 software was used to correct the nanopore sequencing results of the assembled organellar genome of *C.rotundus* [64]. Mitigate (http://dogma.ccbb.utexas.edu/mitofy/) and MFannot software were used to annotate genes [65] and combine the two annotation results for manual correction in the nucleic acid sequence database. The OGDRAW (https://github.com/sjackman/OGDraw/) software was used to draw genome annotation maps [66].

Repetitive sequence and codon preference analysis

Using MISA (http://pgrc.ipk-gatersleben.de/misa/) software for SSR detection of the organellar genome of XFZ01, the parameter threshold was set as follows: the minimum number of replicates for each corresponding repeating unit was 1–10, 2–5, 3–4, 4 – 3, 5 – 3, and 6 – 3 [67]. Advanced software from the online website Tandem Repeats Finder (https://tandem.bu.edu/trf/trf. html/) was used to analyze the tandem repeat (TR) of the organellar genome of XFZ01 with default parameters [68]. REPuter (https://bibiserv.cebitec.uni-bielefeld.de/reputer/) software was used to analyze the palindromic, forward, reverse, and complementary repeat of the organellar genome of XFZ01. The parameter was set to a Hamming distance of three, the maximum number of repetitions was calculated to be 5000, the minimum number of repetitions was calculated to be 30, and filtered with an E-value cutoff of $1e^{-5}$ [69].

A self-encoded Perl script was used to evaluate the codon composition of the XFZ01 organellar genome and the number of codons in each gene. Simultaneously CodonW 1.4.2 (http://codonw.sourceforge.net/) software was used to perform statistical analysis on the ENC, RSCU, average GC content, GC1 (1st codon GC content), GC2 (2nd codon GC content), and GC3 (3rd codon GC content) of the XFZ01 organellar genome.

DNA transfer between the mitogenome and cpgenome

A homology search between the cpgenome (NC050170) and mitogenome was performed using BLASTN to analyze the migrating DNA fragments between the cpgenome and the mitogenome of XFZ01 [70]. The circos package in TBtools was used to visualize the results [71, 72].

RNA editing prediction

Raw transcriptome data were analyzed using FASTP. The parameters were set to default values. Clean data obtained after quality control were compared using BWA-MEM(https://janis.readthedocs.io/en/latest/tools/bioinformatics/bwa/bwamem.html/), and the SAM file obtained after comparison was sorted using SAMtools to obtain the sorted BAM file [73]. Variation detection uses BCF tools to obtain the VCF files [74]. RNA editing events were detected using the Perl script REDO.pl [75].

Phylogenetic evolution and selective pressure analysis

Phylogenetic trees of *C. rotundus* organellar genomes and nine selected plants were constructed. The 9 selected plants include 2 Fabaceae species (*Glycine max* and *Medicago truncatula*), 2 Gramineae species (*Sorghum bicolor andOryza sativa*), 2 Brassicaceae species (*Arabidopsis thaliana* and *Brassica napus*), 1 Arecaceae species (*Phoenix dactylifer*), 1 Solanaceae species (*Solanum tuberosum*), and 1 Cyperaceae species (*C. esculentus*) from NCBI (https://www.ncbi.nlm.nih.gov/) Organellar Genome. The Phylon Suite software (v1.2.2, available at https://dongzhang0725.github.io/) was used to extract 17 mitochondrial and 64 chloroplast PCGs from 10 plants, which were also utilized for splicing and amino acid sequence alignment.

The Ka/Ks values of 17 PCGs among mitogenomes from *C. rotundus* and nine selected plants were determined using PAML (v4.9) [76]. The non-synonymous substitution rate (Ka) and synonymous substitution rate (Ks) were evaluated using the yn00 module. Boxplots and heatmaps were created using GraphPad Prism 8 (GraphPad, Boston, MA, USA) and Majorbio Cloud Platform's online tool (https://cloud.majorbio.com/page/tools/) [77], respectively.

Declarations

Supplementary material

The Supplementary Material for this article can be found online at:

Supplementary Material 1

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Authors'contributions

SX.Y and XR.M executed this study, analyzed data, created charts, and took part in writing the paper; DH.L participated in the analysis of experimental data; and JQ.N and WW.T were the project creators, guiding the experimental design, data analysis, paper writing, and revision. All the authors have read and agreed to the final version of the manuscript.

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Data Availability

The raw sequencing data for the second and third-generation have been deposited in NCBI (https://www.ncbi.nlm.nih.gov/) with accession numbers PRJNA719594, SAMN18613755, SRR14140448, and SRR14140449, respectively. This data can be found in NCBI GenBank under accession numbers OK001344-OK001347 and NC050170.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Figures

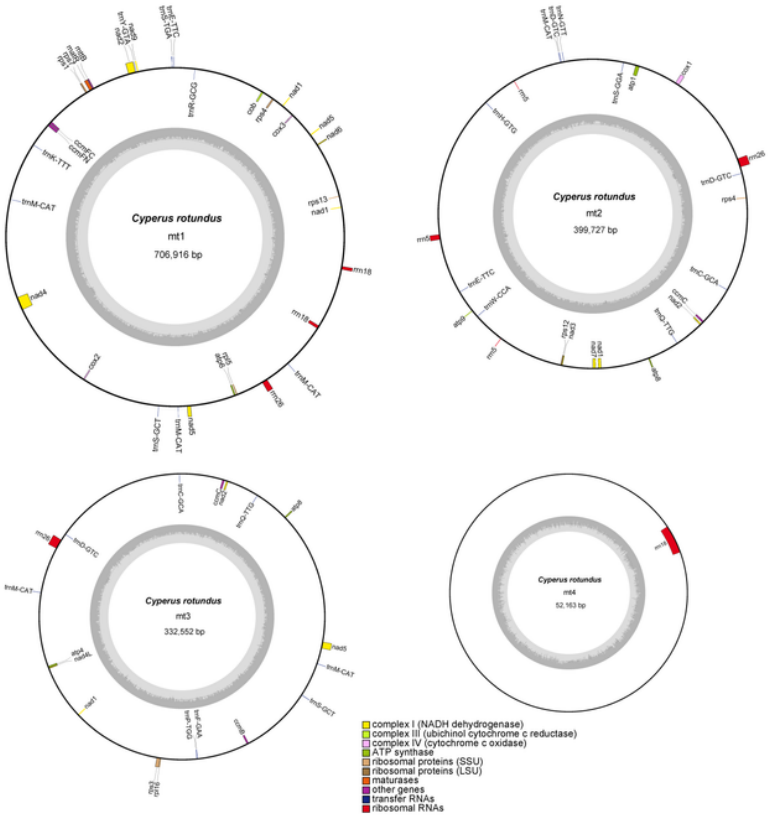

Figure 1

The circular maps of *C. rotundus*mitogenome. The maps display annotated genes for several functional groups that are color-coded on the outer circle to indicate whether transcription is occurring clockwise or counterclockwise. The GC content is displayed as a gray histogram in the inner circle. The gray line in the middle is the 50% threshold line

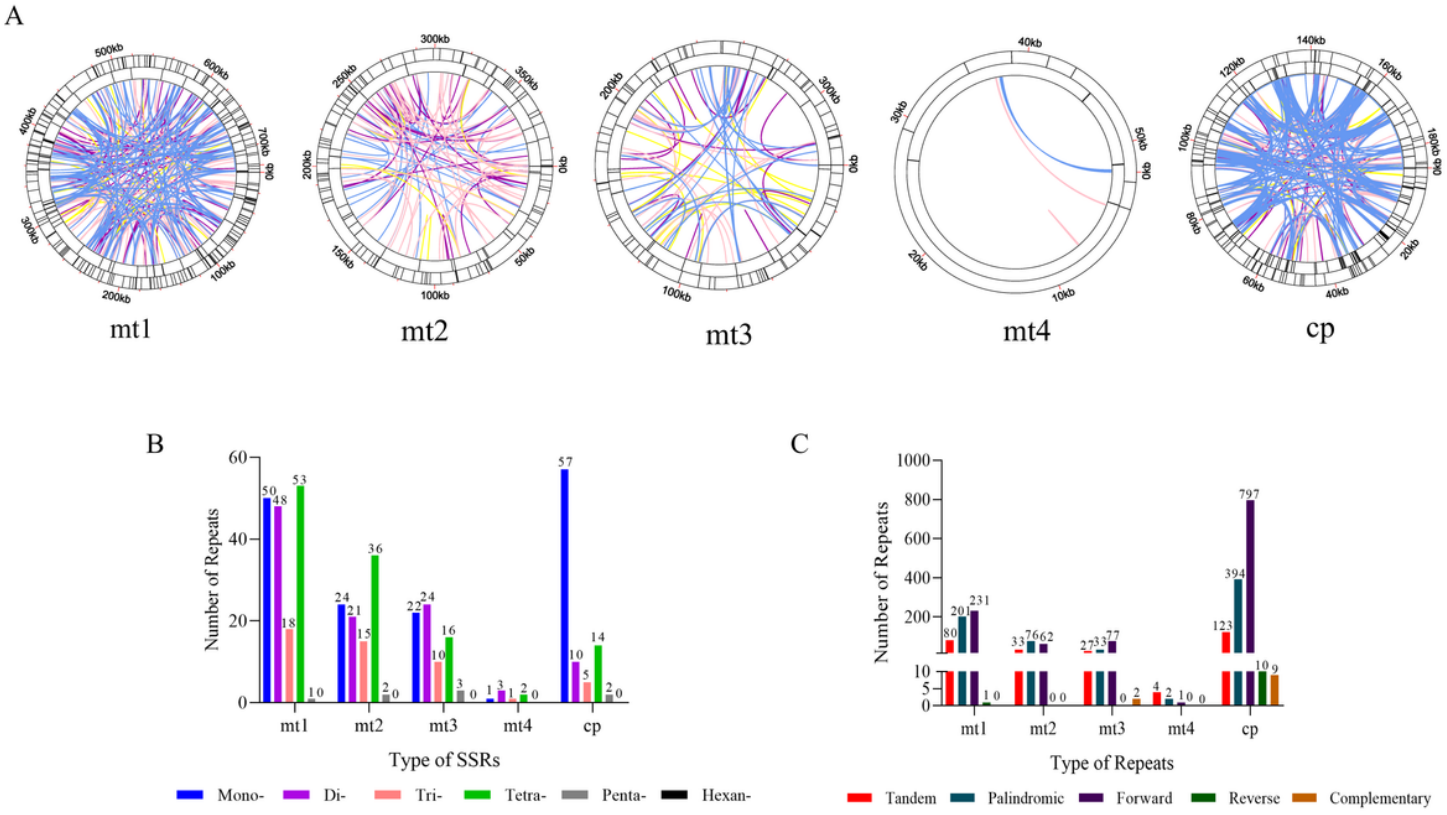

Figure 2

The repeat analysis of *C. rotundus* organellar genomes. (**A**) Repetitive sequences of mitogenome and cpgenome. The inner circle represents interspersed repeats. The two outer circles represent tandem repeats and simple sequence repeats by short bars, respectively. (**B**) The type and number of SSRs. Mono-, Di-, Tri-, Tetra-, Penta-, and Hexan- are abbreviations for Monomeric, Dimeric, Trimeric, Tetrameric, Pentameric, and Hexanmeric, respectively. **(C)** The type and number of repeats

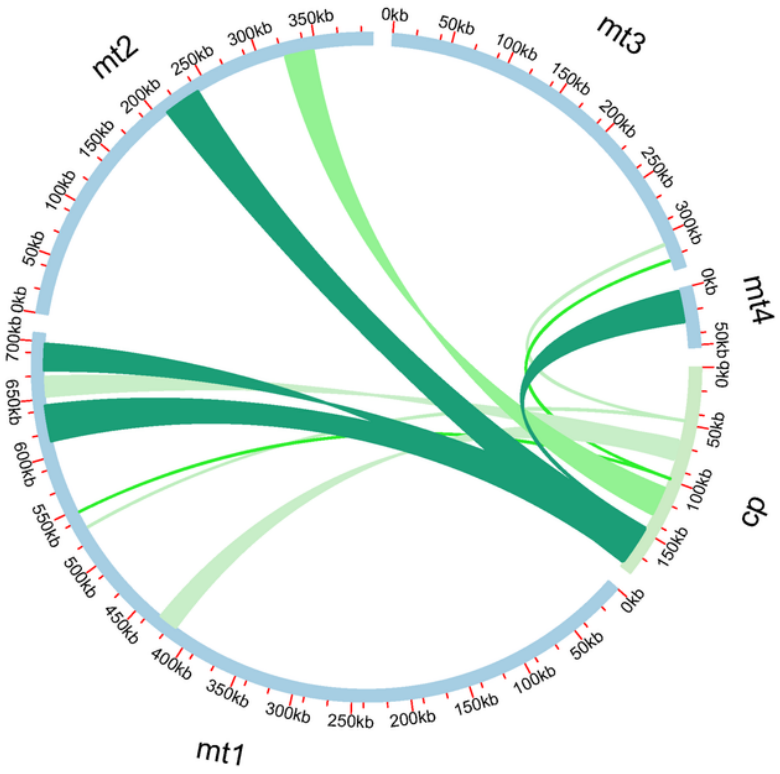

Figure 3

Homologous fragments analysis of *C. rotundus* organellar genomes

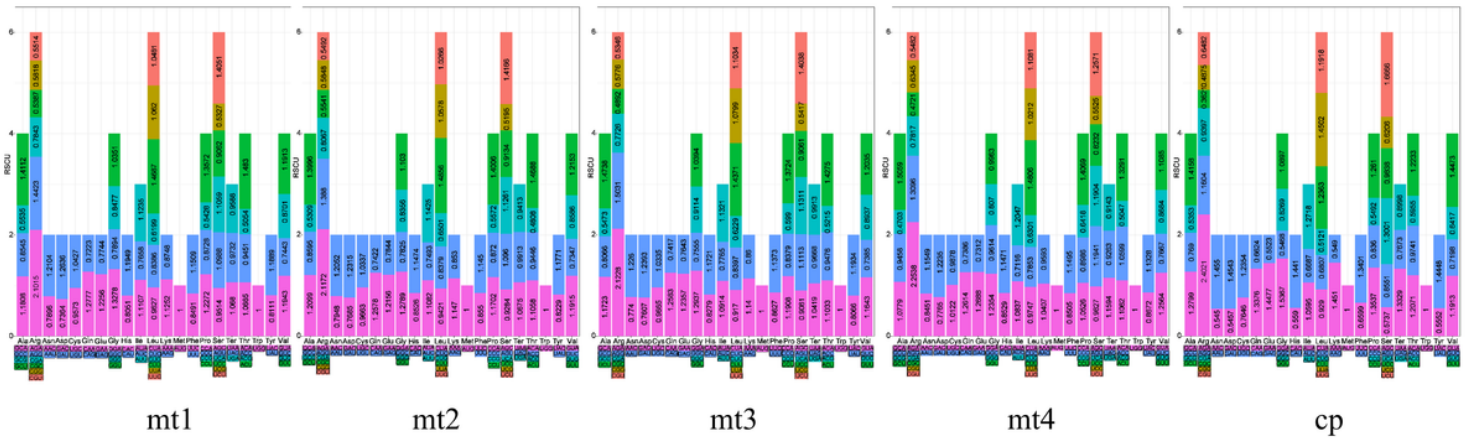

Figure 4

The codon preference of *C. rotundus* organellar genomes

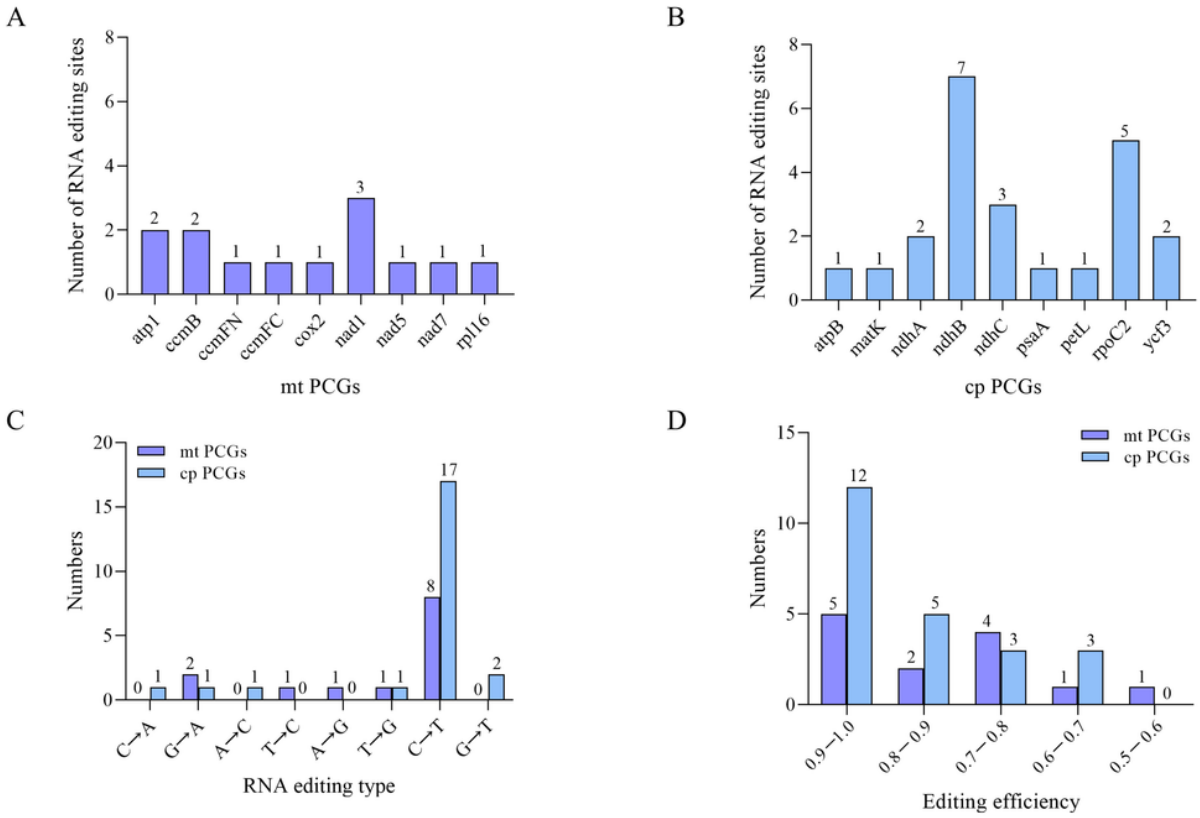

Figure 5

The RNA editing sites in PCGs of *C. rotundus* organellar genomes. (A) The number of RNA editing sites in mitogenome; (B) The number of RNA editing sites in cpgenome; (C) RNA editing type of organellar genomes; (D) RNA editing efficiency of organellar genomes

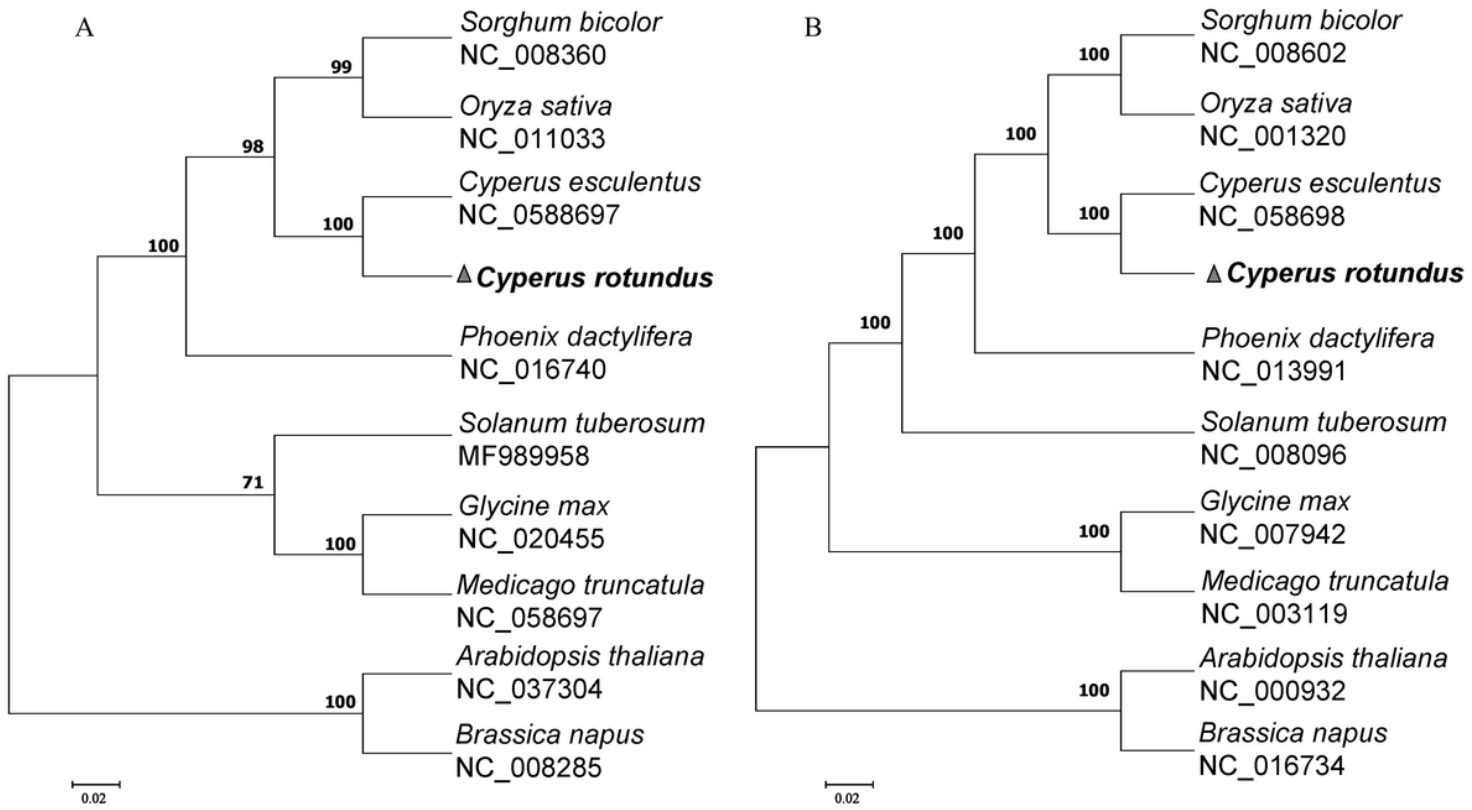

Figure 6

The phylogenetic relationships of *C. rotundus* organellar genomes with other 9 plant species. (A) The mitogenome's phylogenetic trees were built using the amino acid sequences of 17 PCGs. (B) The cpgenome's phylogenetic trees were built using the amino acid sequences of 64 PCGs

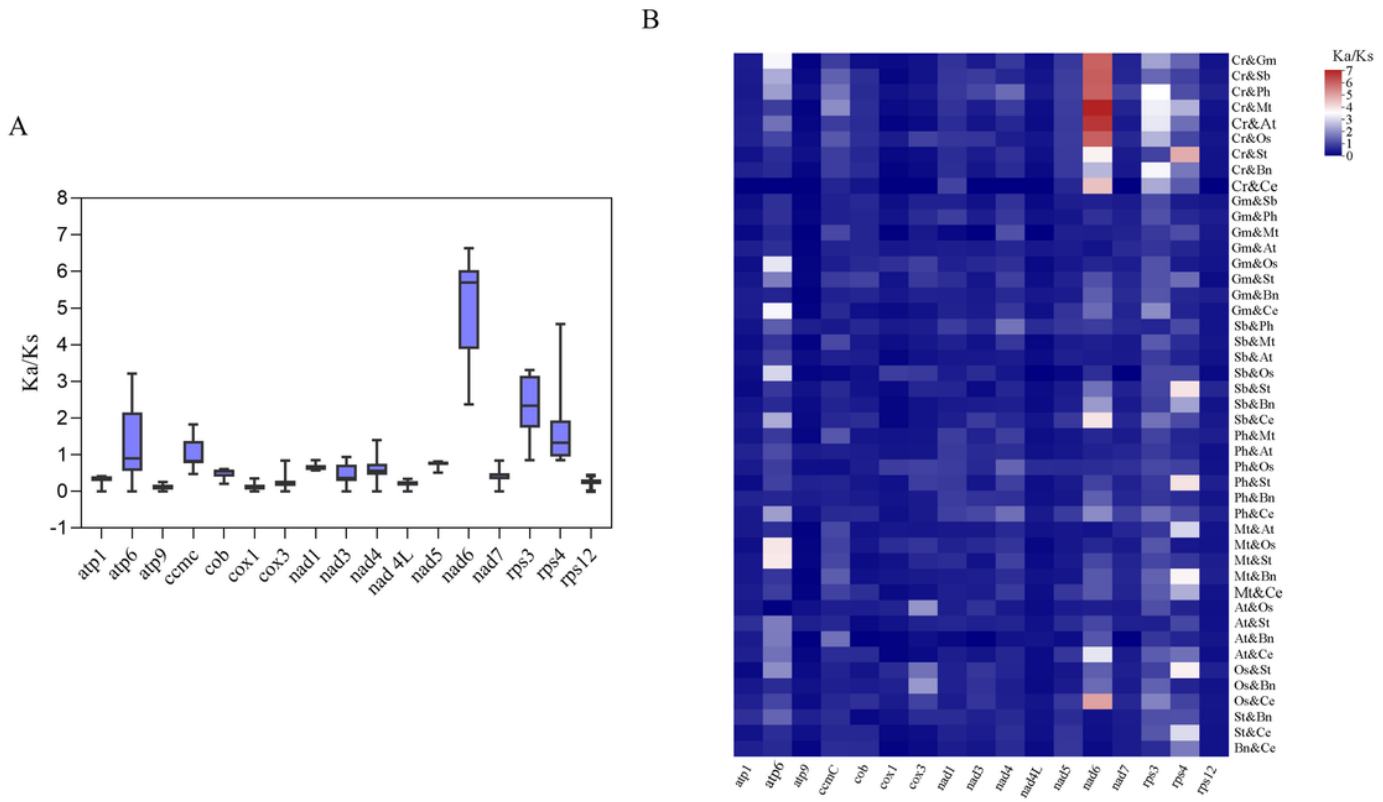

Figure 7

The evolutionary selection pressure analysis of mitogenome. (A) The boxplot of pairwise Ka/Ks values of 17 PCGs of *C. rotundus* mitogenome. (B)The heatmap of pairwise Ka/Ks values between ten species

Supplementary Files

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- SupplementaryMaterial1.xlsx