Sequencing and analysis of the complete mitochondrial genomes of Toona sinensis and Toona ciliata reveal evolutionary features of Toona

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Abstract

Toona is a critical genus in the Meliaceae, and the plants of this group are an asset for both restorative and restorative purposes, the most flexible of which are *Toona sinensis* and *Toona ciliata*. To concentrate on the advancement of mitochondrial genome variety in *Toona sinensis* and *Toona ciliata*, the mitochondrial genomes of the two species were sequenced in high throughput independently, after de novo assembly and annotation to construct a mitochondrial genome map for comparison in genome structure, along with ML phylogenetic analysis with seven other *Toona* relatives. Results show:(1)The mitochondrial genome of *Toona ciliata* is 683,000 bp in length, with 45.40% GC content and circular structure, and contains 38 protein genes plus 33 transfer RNA genes, of which 33 tRNAs encode 20 amino acids. AT preference exists for MtDNA, TTT is the most frequently encountered codon, appearing 413 times, as well as the largest codon corresponding to The codon encoding the equivalent of Phe, which had the maximum content. (2)Mitochondrial genome of *Toona sinensis* is 638482bp in entire length, with 45.56% GC content and a circumscribed architecture, it encodes 38 protein genes and 34 transfer RNA genes, among 34 tRNAs embodying 20 amino acids. AT is preferred by mtDNA, whereas TTT, which is utilized 412 times quite often and seems to have the maximum Phe concentration, is the most frequently employed codon. (3)Both the mitochondrial genomes of *Toona sinensis* and *Toona ciliata* are highly preserved in terms of structural and functional genes, while the main variability is reflected in the length of tRNA and the number of genes. (4) In the ML evolutionary tree, *Toona sinensis* and *Toona ciliata* clustered individually into a small branch with 100% support, reflecting two species of *Toona* are very similarly related to each other. This research provides a basis for the exploitation of *Toona sinensis* and *Toona ciliata* in terms of medicinal, edible, and timber resources to avoid confusion; at the same time, it can explore the evolutionary relationship between the *Toona* and related species, which does not only have an important practical value, but also provides a theoretical basis for future hybrid breeding of forest trees, molecular markers, and evolutionary aspects of plants, which has great scientific significance.

Introduction

Toona plants have magnificent material, straight surface, and radiance, turning into the predominant furnishings and inside adornment wood, known as "Chinese mahogany", which is greatly esteemed by individuals(Bangyu 1995; Chengjing ; Guoqian and Shiman 1991; Liwen et al. 2014; Zhenfu, Ruilong, and Jieling 2003). *Toona sinensis* and *Toona ciliata* have the most noteworthy application in the *Toona*. *Toona sinensis (A. Juss) Roem* is a unique species of vegetable in China, its young shoots and leaves are crisp and juicy, fragrant and unique in flavor, it is a traditional and valuable woody vegetable that our people like to eat, and is also a local product for foreign trade export(Fangren and Youwang 2005; Libo et al.). *Toona sinensis* is not only an excellent vegetable but also a natural green nutritious food of medicinal and food origin. It has somewhat high happiness of flavone and other pharmacologically dynamic mixtures(Lixin et al. 2015; Yang et al. 2016). *Toona ciliata* is a Grade II safeguarded plant, an important timber tree, and a therapeutic plant that has acquired broad consideration as of late(Dongmei et al. 2015; Duanmu 1996; Li et al. 2018; Li et al. 2015). The roots, stems, and leaves of *Toona ciliata* can be utilized as medication and have successful restorative properties(Lucchese et al. 2019; Nassur et al. 2013; Parthiban et al. 2019). The monetary worth of this variety is quite high, and it is generally utilized and has extraordinary potential for advancement and utilization(Ribeiro et al. 2014; Wei et al. 2020; Yadong et al. 2022; Zhuchou et al. 2022).

Mitochondria are organelles in higher plant cells with a semi-autonomous genetic system that provides the majority of the energy required for cellular and other life activities(Baolong 2012; Guodong 2016; Guohong, Na, and Jianrong 2012; Hao et al. 2021). Mitochondria are particularly important in the study of the origin and evolution of living things. The mitochondrial DNA (mtDNA) is a genetic material found outside the nucleus that is normally a double-stranded circular molecule with a covalent closure(Hisano et al. 2016; Huo et al. 2016; Jongsun et al. 2020). Advanced plants have the

largest mitochondria of any known higher organism species, ranging from 200 kb to 2400 kb(S. et al. 2021; Small and Wendel 1999; Wanqi 2020).

Plant mitochondrial genomes have been increasingly studied and more and more mitochondrial genomes have been sequenced in recent years, which is very important for studying the diversity of biological phenotypes, functional diversity, as well as species evolution. This is critical for understanding biological phenological diversity, functional diversity, and the emergence of new functions during species evolution(Wenlong, Fu, and Guohong 2019; Woojong et al. 2021).

Even though *Toona* plants have a long history of cultivation in China, most studies have been limited to chemical pathology, physiology, biochemistry, introduction, and breeding, with little research done on its origin, taxonomy, cytogenetics, and so on. There are still some issues with *Toona* classification, such as interspecific hybridization, that need to be addressed(Changxun, Dechun, and Debing 2001; Chunhua et al. 2022). Furthermore, *Toona* plants have a geographically dispersed distribution in China, resulting in a scarcity of natural forests and susceptibility to natural and anthropogenic breakage, and *Toona ciliata* has now been classified as an endangered plant(Qiang 2016; Ruyu, Guangyou, and Jianmin 2005; Tieshan et al. 2000; Jun et al. 2013).

Subsequently, this review, given mitochondrial near genomic examination through trend-setting innovations, for example, sub-atomic sequencing of mitochondrial DNA, makes it conceivable to concentrate on *Toona* further top to bottom according to a minuscule viewpoint notwithstanding plainly visible morphological characterization and makes the preservation of excellent hereditary assets of the imperiled species *Toona ciliata*, determination and reproducing of good species, and advancement and usage with significant hypothetical and functional importance.

1. Materials And Methods

1.2. Plant material, DNA extraction, and Library construction

While *Toona sinensis* was acquired from Pingxiang, Guangxi, *Toona ciliata* was obtained from Baoshan, Yunnan (Longitude: 106.75 E, Latitude: 22.12 N.) Before this investigation, both species completed seedling trials and were found to be suitable for cultivation in Guangzhou, Guangdong. (Note: WeiZhou conducted a detailed identification of the plant material. The seed trial forest is situated near the South China Agricultural University's teaching and research facility in Guangzhou, China, at N23°16' and E113°37'.)

High-quality total DNA is the primary prerequisite for obtaining the whole mitochondrial genome sequence. Fresh leaves of *Toona sinensis* and *Toona ciliata* were taken and whole genome DNA was extracted by the CTAB (Irfan et al. 2013)method. high-quality genome DNA was extracted and quality checked for purity, concentration, and integrity using Nanodrop (Qian et al. 2017; Guodong 2016), 1% (w/v) agarose gel electrophoresis. DNA samples that passed the electrophoresis test were randomly broken into fragments of approximately 350 bp in length using a Covaris ultrasonic fragmentation machine(Tim et al. 2014). After processing, the DNA fragments were subjected to end repair, A-tail addition, sequencing junction addition, purification, PCR amplification, and other steps to complete the entire library preparation. After the library was constructed, the initial quantification was performed using Qubit 3.0, and the library was diluted to 2 ng/ul. The insert size (insert size) of the library was then detected using Agilent 2100(J et al. 2000; Liu, Padmanaban, and Etteldorf 2016; Wanqi 2020), after the insert size met the expectation, the effective concentration of the library was quantified using Q-PCR. After the inserts met the expectation, the effective concentration of the library was accurately quantified by Q-PCR (Yuxia 2013)to ensure the quality of the library. After the libraries passed the test, they were sent to Guangzhou Ruike Gene Technology Co.

1.3. Sequencing, Assembly, and Annotation

Utilizing the Illumina HiSeq 4000 High-throughput Sequencing Platform, qualified DNA libraries were sequenced. Once the sequencing was completed, the sequenced data were spliced into the mitochondrial genome. The reads with low sequencing quality (< 40bp in length) were filtered by Trimmomatic (M, Marc, and Bjoern 2014), the overlapping reads were filtered out by Blast to obtain Clean Data, and the sequencing data were analyzed by 15-mer using K-mer software to obtain high-quality reads. Assembly was performed using SOAP denovo (Minjie et al. 2013)assembly software. The preliminary assembly results were optimized and holes were filled using krskgf and gapclose (Xu et al. 2020)software to obtain the specific assembly results.

The complete mitochondrial genome sequence was annotated utilizing CPGAVAS (Chang et al. 2012)software together with DOGMA (K, K, and L 2004)software. Transfer RNA (transfer RNA, tRNA) genes were identified along with manual correction employing tRNAscan-SE (M and R 1997) software. The BLAST (Liping 2021)search method was performed to align (Federico, Rafael, and David 2005; Koichiro, Glen, and Sudhir 2021; Tang et al. 2015)and validate (Kersten et al. 2016)the information sites such as gene boundaries, intron, exon, and coding regions.

1.4. NCBI submission

The annotated genome sequences were submitted to NCBI according to the requirements, resulting in the definitive accession numbers *Toona sinensis* (GenBank: OM574631.1) and *Toona ciliata* (GenBank: OM574630.1).

1.5. Superior Mitochondrial Genome Analysis

1.5.1. Structure and composition

Mitochondria were mapped by OGDRAW vl.2 (Organellar Genome DRAW) (Marc, Oliver, and Ralph 2007) online website (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html). The circular structure of the genome sequence was mapped. The base content of the mitochondrial genome was calculated using Editseq (C and P 1997)software to obtain the ratio of A, T, C, G, and GC content respectively.

1.5.2. Frequency of codon usage

Considering the formula mentioned in Sharp PM literature(M and H 1987), the utilization of relative equivalent codon use (RSCU) was examined utilizing CodonW (M and H 1987; P et al. 2015)software.

1.5.3. Phylogenetic tree analysis

Species with complete mitochondrial genome arrangements and explanations in direct relation to the objective species were downloaded from NCBI for phylogenetic tree development. The protein-coding qualities of the mitochondrial genomes of the species checked for phylogenetic tree development were chosen independently (by and large, the coding qualities normal to all species were gathered for tree development). MUSCLE v.3.8.31 (http://www.drive5.com/muscle/) programming was utilized to play out the correlation of individual qualities among various species. And afterward, the qualities of every species after the examination were coordinated in a specific request couple to shape the protein-coding quality succession set of that species. Afterward, the groupings were utilized for the procedure investigation. Nucleic corrosive model testing was performed against chosen grouping DNA utilizing jModelTest2.1.7 (David 2009, 2008)(https://code.google.com/p/jmodeltest2/) and amino corrosive model test with Prottest 3.2(2)(Diego et al. 2011; Federico, Rafael, and David 2005) (https://code.google.com/p/prottest3/). The base worth of AIC (Akaike Information Criterion)(David 2008; Ehsan et al. 2022) was chosen as the best model for tree development. RAxML 8.1.5(Dimitri et al. 2021) (https://sco.h-its.org/exelixis/web/programming/raxml/index.html) programming, Maximum Likelihood strategy (ML technique: Maximum Likelihood technique)(Koichiro, Glen, and Sudhir 2021; Wenlong, Fu, and Guohong 2019; Baolong 2012; Wanqi 2020) was utilized to develop the phylogenetic tree, and Bootstrap esteem was set to 1000.

2. Results & Analysis

2.1. Genome Features

The total mitochondrial genome length of *T. ciliata* was 683,000 bp, the composition of bases was A (27.31%), T (27.29%), C (22.56%), and G (22.85%), and the C + G content was 45.40%. The size of the *T. sinensis* mitochondrial genome was 638482 bp, and its base makeup was A (27.35%), T (27.09%), C (22.79%), and G (22.76%), with a C + G content of 45.56%. All of them have a circumferential mitochondrial genome construction, where their longest gene is the rrn26 gene in the transfer RNA, measuring 3116 bp (Table 1; Fig. 1).

Reverse transcription is indicated by genes outside of the circles, and clockwise transcription is indicated by genes inside the circles. The two IR regions are represented by the thick black line on the outside circle. The GC content is represented by the inner nucleus' dark gray graph.

Table 1
Results of mt DNA genome sequence analysis of two plants.

	Toona_ciliata		*Toona_sinensis*	
Type	**Size**	**Proportion**	**size**	**Proportion**
A content	186,538	27.31%	174,629	27.35%
T content	186,361	27.29%	172,983	27.09%
G content	156,039	22.85%	145,343	22.76%
C content	154,062	22.56%	145,527	22.79%
Total content	683,000		638,482	
G + C content	310,101	45.40%	290,870	45.56%
longest gene	3,116	50.77%	3,116	50.77%
Note: Both mitochondria of the longest gene are 26S-rRNA, 50. The GC content of the gene is 50.77%.				

2.2. Functional gene

2.2.1. Gene encoding protein

T.ciliata encodes 71 genes while *T.sinensis* encodes 72 genes. Protein-coding genes of both *Toona* plants are consistent in frequency, types, and measurements (Table 2), whereas the predominant divergence is in tRNAs, with *T. ciliata* encoding 33 tRNAs and *T. sinensis* encoding 34 (Table 3).

Table 2
Gene content in mitochondrial genomes of *T.ciliata* and *T.sinensis.*

Product group	Gene	*T.ciliata*		*T.sinensis*	
			size(bp)		size(bp)
Complex I	*nad1*	+	978	+	978
	nad2	+	1467	+	1467
	nad3	+	357	+	357
	nad4	+	1485	+	1485
	nad5	+	1989	+	1989
	nad6	+	618	+	618
	nad7	+	1188	+	1188
	nad4l	+	303	+	303
	nad9	+	573	+	573
Complex II	*sdh3*	+	369	+	369
	sdh4	+	426	+	426
Complex III	*cob*	+	1182	+	1182
Complex IV	*cox1*	+	1584	+	1584
	cox2	+	783	+	783
	cox3	+	798	+	798
Complex V	*atp1*	+	1530	+	1530
	atp4	+	597	+	597
	atp6	+	720	+	720
	atp8	+	486	+	486
	atp9	+	300	+	300
Cytochrome c	*ccmB*	+	621	+	621
	ccmC	+	753	+	753
	ccmFC	+	1476	+	1476
	ccmFN	+	1797	+	1797
Ribosome Protein	*rps1*	+	606	+	606
	rps3	+	1692	+	1692
	rps4	+	1692	+	1692
	rps10	+	333	+	333
	rps12	+	378	+	378

Product group	Gene	*T.ciliata*		*T.sinensis*	
	rpl2	+	1035	+	1035
	rpl16	+	558	+	558
	rpl5	+	555	+	555
	rpl10	+	489	+	489
Ribosome RNA	*rrn5*	+	119	+	119
	rrn18	+	1954	+	1954
	rrn26	+	3116	+	3116
tRNA(Detail in Table 3)		33		34	
other ORF	*matR*	+	1917	+	1917
	mttb	+	786	+	786
total		71		72	

Employing NCBI-BLAST analysis, 38 genes encoding proteins were obtained on the mitochondrial genomes of both *T. ciliata* and *T. sinensis*. We categorized the protein-encoding genes into the following eight categories according to their gene functions (Table 2): including Complex I genes (*nad1, nad2, nad3, nad4, nad4L, nad5, nad6, nad7*, and *nad9*) involved in the synthesis of NADH deaminase subunits; Complex II genes (*sdh3* and *sdh4*) participated in the synthesis of cytochrome b precursor subunits ; Complex III gene (*cob*) implicated in the synthesis of the cytochrome C oxidase subunit; Complex IV genes (c*ox1, cox2*, and *cox3*) Complex V genes (*atp1, atp4, atp6, atp8*, and *atp9*), associated with the synthesis of ATP synthase subunits Cytochrome c biosynthetic genes (*ccmB, ccmC, ccmFC* and *ccmFN*) engaged in the synthesis of cytochrome C synthase subunits Ribosome protein genes synthesized by ribosome protein synthesis genes (*rps1, rps3, rps4, rps10, rps12, rpl2, rpl5, rpl10* and *rpl16*) The ribosomal RNA genes (*rrn5, rrn18* and rrn26) as well as the *matR* gene (encoding a maturation-like enzyme) and the *mttB* gene (encoding a transporter).

2.2.2. Gene encoding tRNA

Utilizing tRNAscan-SE, 33 and 34 genes encoding transfer RNAs were identified on the mitochondrial genomes of *T. ciliata* and *T. sinensis*, separately.

In the *T. ciliata* gene, a total of 33 tRNAs encode 20 amino acids ranging from 66 bp-88 bp in length. five of these tRNASers, LeU, Gly, Gly, Cys, Arg, and Lys each have two tRNAs encoding, Met and Pro are distributed with three tRNAs encoding, and the remaining amino acids all have one tRNA Editor. In contrast to *T. ciliata, T. sinensis* has 34 tRNAs encoding 20 amino acids, ranging from 63–167 bp in length. compared to *T. ciliata, T. sinensis* has 2 fewer tRNAs encoding Cys and Arg, but 3 more tRNAs encoding Pro, ILe, and Glu (Table 3).

Table 3
tRNA present in the *T.ciliata* and *T.sinensis* mitochondrial genome.

Table 3 *tRNA present in the T.ciliata and T.sinensis mitochondrial genome.*										
	T.ciliata					T.sinensis				
Type	**Number**	**Anti-codon**	**Size (bp)**	**GC_**	**One Letter code**	**Number**	**Anti-codon**	**Size(bp)**	**GC_**	**One Letter code**
Ser	5	TGA	67	50.75%	S	5	CGA	67	47.76%	S
		GGA	82	42.68%	S		GGA	82	42.68%	S
		GCG	88	51.14%	S		TGA	88	51.14%	S
		GCT	88	46.59%	S		GCT	88	46.59%	S
		GGA	72	51.72%	S		TGA	167	49.10%	S
Leu	2	CAG	73	46.58%	L	2	GAG	75	46.67%	L
		CAG	69	49.28%	L		CAG	73	46.58%	L
Met	3	CAT	72	59.72%	M	3	CAT	77	42.86%	M
		CAT	77	42.86%	M		CAT	73	43.84%	M
		CAT	74	43.84%	M		CAT	72	59.72%	M
Gln	1	TTG	74	47.22%	Q	1	TTG	72	47.22%	Q
Gly	2	GCC	73	54.05%	G	2	GCC	63	55.56%	G
		TGT	76	43.42%	G		GCC	74	54.05%	G
Asp	1	GTC	84	62.16%	D	1	GTC	74	62.16%	D
Asn	1	GTT	72	52.78%	N	1	GTT	72	52.78%	N
Cys	2	GCA	72	51.39%	C	1	GCA	72	51.39%	C
		GCA	73	52.05%	C					
Pro	3	CGG	74	31.08%	P	4	TGG	72	48.61%	P
		/	77	49.35%	P		TGG	77	49.35%	P
		CCC	75	54.67%	P		CGG	74	31.08%	P
							TGG	75	54.67%	P
Trp	1	TGG	74	51.35%	W	1	CCA	74	51.35%	W
Ala	1	CCA	66	46.97%	A	1	GGC	87	55.17%	A
His	1	GGC	75	60.00%	H	1	GTG	75	60.00%	H
Thr	1	GTG	81	45.68%	T	1	TGT	81	45.68%	T
Arg	2	TTT	74	41.89%	R	1	GCG	74	41.89%	R
		CCG	75	58.67%	R					

Table 3 *tRNA present in the T.ciliata and T.sinensis mitochondrial genome.*

Val	1	GAC	87	36.78%	V	1	TAC	81	40.74%	V
Phe	1	GAA	74	45.95%	F	1	GAA	74	45.95%	F
Ile	1	TGG	73	47.95%	I	2	TAT	73	47.95%	I
							TAT	73	47.95%	I
Lys	2	TAT	74	47.30%	K	2	TTT	74	47.30%	K
		TTC	75	48.00%	K		TTT	75	48.00%	K
Glu	1	TGA	72	50.00%	E	2	TTC	72	50.00%	E
							TTC	71	50.70%	E

Note:/ Indicates unknown or not found

2.3. Codon Usage bias

RSCU (Relative Synonymous Codon Usage)(P et al. 2015) is a measure of relative synonymous codon usage, indicating the proportion of a given synonymous codon usage among all synonymous codons. The mitochondrial genomes of *T. ciliata* and *T. sinensis* have a codon usage bias for all amino acids except for the Trp of only one codon, TGG.

The codon TTT was the most frequently accessed codon in the mitochondrial protein-coding genes of *T. ciliata* and *T. sinensis*, with the second commonest codon being ATT and the third being TTC. The termination codon TAG was the least frequently addressed codon, being exclusively indexed six and five occasions respectively (Table 4).

Table 4
Statistical table of RSCU values for *T.ciliata* and *T.sinensis*

	T.ciliata				*T.sinensis*			
Amino acid	**Codon**	**Number**	**Frequency (%)**	**RSCU**	**Codon**	**Number**	**Frequency (%)**	**RSCU**
Ala	GCT	268	2.529256323	1.60960961	GCT	267	2.523390984	1.608433735
Ala	GCA	159	1.500566251	0.954954955	GCA	158	1.493242605	0.951807229
Ala	GCC	153	1.44394111	0.918918919	GCC	153	1.445988092	0.921686747
Ala	GCG	86	0.811627029	0.516516517	GCG	86	0.81277762	0.518072289
Arg	AGA	172	1.623254058	1.461756374	AGA	173	1.635006143	1.472340426
Arg	CGA	155	1.462816157	1.317280453	CGA	155	1.464889897	1.319148936
Arg	CGT	137	1.292940732	1.164305949	CGT	137	1.294773651	1.165957447
Arg	AGG	93	0.877689694	0.790368272	AGG	93	0.878933938	0.791489362
Arg	CGG	79	0.745564364	0.671388102	CGG	78	0.7371704	0.663829787
Arg	CGC	70	0.660626652	0.59490085	CGC	69	0.652112277	0.587234043
Asn	AAT	224	2.114005285	1.333333333	AAT	225	2.126453076	1.339285714
Asn	AAC	112	1.057002643	0.666666667	AAC	111	1.049050184	0.660714286
Asp	GAT	226	2.132880332	1.36969697	GAT	226	2.135903979	1.36969697
Asp	GAC	104	0.981502454	0.63030303	GAC	104	0.982893866	0.63030303
Cys	TGT	94	0.887127218	1.160493827	TGT	94	0.888384841	1.175
Cys	TGC	68	0.641751604	0.839506173	TGC	66	0.623759569	0.825
Gln	CAA	217	2.04794262	1.466216216	CAA	217	2.050845856	1.466216216
Gln	CAG	79	0.745564364	0.533783784	CAG	79	0.746621302	0.533783784
Glu	GAA	311	2.935069838	1.385300668	GAA	311	2.939230697	1.388392857
Glu	GAG	138	1.302378256	0.614699332	GAG	137	1.294773651	0.611607143
Gly	GGA	266	2.510381276	1.453551913	GGA	266	2.513940081	1.457534247
Gly	GGT	231	2.18006795	1.262295082	GGT	230	2.173707589	1.260273973
Gly	GGG	134	1.264628162	0.732240437	GGG	134	1.266420943	0.734246575
Gly	GGC	101	0.953189883	0.551912568	GGC	100	0.945090256	0.547945205
His	CAT	190	1.793129483	1.526104418	CAT	190	1.795671487	1.526104418
His	CAC	59	0.556813892	0.473895582	CAC	59	0.557603251	0.473895582
Ile	ATT	346	3.265383163	1.284653465	ATT	348	3.288914091	1.288888889
Ile	ATC	240	2.265005663	0.891089109	ATC	240	2.268216615	0.888888889

	T.ciliata				*T.sinensis*			
Ile	ATA	222	2.095130238	0.824257426	ATA	222	2.098100369	0.822222222
Leu	TTA	294	2.774631937	1.48609941	TTA	294	2.778565353	1.488607595
Leu	CTT	250	2.359380898	1.263689975	CTT	247	2.334372933	1.250632911
Leu	TTG	229	2.161192903	1.157540017	TTG	230	2.173707589	1.164556962
Leu	CTA	181	1.70819177	0.914911542	CTA	180	1.701162461	0.911392405
Leu	CTG	119	1.123065308	0.601516428	CTG	119	1.124657405	0.602531646
Leu	CTC	114	1.07587769	0.576242628	CTC	115	1.086853795	0.582278481
Lys	AAA	261	2.463193658	1.186363636	AAA	261	2.466685568	1.183673469
Lys	AAG	179	1.689316723	0.813636364	AAG	180	1.701162461	0.816326531
Met	ATG	281	2.65194413	1	ATG	280	2.646252717	1
Phe	TTT	413	3.897697244	1.12995896	TTT	412	3.893771855	1.128767123
Phe	TTC	318	3.001132503	0.87004104	TTC	318	3.005387014	0.871232877
Pro	CCT	207	1.953567384	1.5	CCT	206	1.946885928	1.500910747
Pro	CCA	149	1.406191015	1.079710145	CCA	148	1.398733579	1.078324226
Pro	CCC	110	1.038127595	0.797101449	CCC	109	1.030148379	0.79417122
Pro	CCG	86	0.811627029	0.623188406	CCG	86	0.81277762	0.626593807
Ser	TCT	213	2.010192525	1.395196507	TCT	213	2.013042246	1.395196507
Ser	AGT	171	1.613816535	1.120087336	AGT	173	1.635006143	1.133187773
Ser	TCA	165	1.557191393	1.080786026	TCA	164	1.54994802	1.074235808
Ser	TCC	138	1.302378256	0.903930131	TCC	139	1.313675456	0.910480349
Ser	TCG	132	1.245753114	0.864628821	TCG	130	1.228617333	0.851528384
Ser	AGC	97	0.915439789	0.635371179	AGC	97	0.916737548	0.635371179
Stp	TAA	18	0.169875425	1.588235294	TAA	18	0.170116246	1.588235294
Stp	TGA	10	0.094375236	0.882352941	TGA	11	0.103959928	0.970588235
Stp	TAG	6	0.056625142	0.529411765	TAG	5	0.047254513	0.441176471
Thr	ACT	185	1.745941865	1.335740072	ACT	184	1.738966071	1.330922242
Thr	ACC	143	1.349565874	1.032490975	ACC	143	1.351479066	1.034358047
Thr	ACA	140	1.321253303	1.010830325	ACA	140	1.323126359	1.012658228
Thr	ACG	86	0.811627029	0.620938628	ACG	86	0.81277762	0.622061483
Trp	TGG	166	1.566628917	1	TGG	165	1.559398923	1
Tyr	TAT	259	2.444318611	1.510204082	TAT	258	2.438332861	1.50877193
Tyr	TAC	84	0.792751982	0.489795918	TAC	84	0.793875815	0.49122807

	T.ciliata				*T.sinensis*			
Val	GTA	193	1.821442054	1.17325228	GTA	194	1.833475097	1.179331307
Val	GTT	192	1.81200453	1.167173252	GTT	191	1.805122389	1.161094225
Val	GTG	153	1.44394111	0.930091185	GTG	154	1.455438994	0.936170213
Val	GTC	120	1.132502831	0.729483283	GTC	119	1.124657405	0.723404255
Remark: Frequency (%): codon usage frequency.								

2.4. ML Phylogeny Analysis

Aiming to ascertain the evolutionary status of *T. ciliata* and *T. sinensis* in the plant system, we downloaded the mtDNA sequences of the same ORDER relatives that have published their mtDNA sequences on NCBI. Six Anacardiaceae species, five Sapindaceae species, three Rutaceae species, two Nitrariaceae species, and two Meliaceae species, *Toona ciliata* (OM574630) and *Toona sinensis* (OM574631), for a total of 18 tree species. The outgroup for the mitochondrial genome was *Morus notabilis* (NC 041177.1), and an evolutionary tree was constructed using the maximum likelihood method using the software MEGA 11(Koichiro, Glen, and Sudhir 2021).In the likelihood ML phylogenetic tree (Fig. 2), a total of 15 nodes were formed, nine of which had 100 percent support, except for the large branch of Anacardiaceae and Nitrariaceae, which had 69 percent support, and *Xanthoceras sorbifolium* (MK333231.1) and *Sapindus mukorossi* (MT806100.1), which formed a minor branch with only 56 percent support, but all the other nodes had no less than 93% support.

3. Discussion

3.1. Mitochondrial Structure & Genetic Comparison

In terms of GC content, gene content, and genetic codon usage preference, the numerical mitochondrial genomes of *T. ciliata* and *T. sinensis* were well conserved. *T. ciliata* and *T. sinensis* had GC values of 45.40 and 45.56 percent each, respectively, and AT contents of 54.6 and 54.44 percent, with an A/T preference. The two *Toona* plants have 38 protein-coding genes and three rRNA genes in common, making their mitochondrial genomes practically identical.

Within the two *Toona*, genes in complexes I, III, IV, and V, ribosomal RNA genes, protein translocator, and intron maturation enzyme genes are conserved between mitochondrial genomes. These encoded genes are mainly concerned with the synthesis of ATP synthase subunits, cytochrome C synthesis, and ribosomal protein synthesis. This provides the theoretical conditions for the exploration of the mechanisms and pathways of metabolite synthesis reactions including respiration and other related metabolites between the two species.

It is speculated that in *T. ciliata*, tRNAs from either the chloroplast or the nucleus would be involved in amino acid translocation to achieve the required number of amino acids for life, since there are 33 tRNA genes in *T. ciliata* and 34 tRNAs in *T. sinensis*, although they encode the same 20 amino acids.

In addition, the codons specifying tRNAs of the two plants are not identical regardless of the determination of the endogenous amino acids, the sizes of the coding genes also differ, thus revealing the existence of a superb concatenation of codons in the mitochondrial genome between the two plants in the Toona, and the codons encoding the simultaneous presence of the first amino acid are called synonymous codons.

Similarly, the type of codons and amino acid species encoded by *T. ciliata* and *T. sinensis* were completely identical, there was almost no discrepancy in codon frequency, generally, less than 0.01, and the difference was dominant in the number of codons used, which only differed by one number. The difference in RSCU values between the two plants was very slight, the majority of the values were the same, differing by less than 0.02.

The determination of RSCU values illustrates that *T. ciliata* and *T. sinensis* are remarkably conserved in terms of codon preference. *T. ciliata* and *T. sinensis* exhibit a mitochondrial preference for A or T. Corresponding to the codon usage bias, the amino acid composition of their protein-coding genes are marked by arginine, isoleucine, alanine, and phenylalanine The content of Stp was higher and the content of Stp was minimum (Table 4).

Heritable traits were drawn directly from tRNA, which is hooked up to the horizontal transfer of genetic material between the mitochondria and the nucleus and chloroplasts, in the mitochondrial genomes of the two plants. The nucleus, chloroplasts, and mitochondria frequently exchange genetic information during genetic evolution, and their DNA typically undergoes genetic drift. As a side effect, mitochondrial DNA exhibits the phenomenon of chloroplast-derived DNA or nuclear-derived DNA, and hybrid DNA appears; among several hybrid DNAs, tRNA has the highest proportion(Yidong et al. 2001; Zhenhua 2008). The similarity between mitochondrial genes and other genetic material has not yet been explored in depth, hence it is questionable whether the evolution between the two *Toona* genera is linked to gene sequence migration analysis, and the loss or transfer of particular genes.

This analysis serves as a foundation for additional investigations into the physiological behaviors correlated to amino acids, the nutritional composition associated with codon substitution bias, and the amino acid composition of protein-coding genes.

3.2. Systematic Evolution

T.ciliata and *T. sinensis* have similar morphological characters and cultivate in similar environments, so the traditional morphological taxonomy considers the two plants to be cloplantssely related(Jiming et al. 2017; Liao et al. 2016). In the phylogenetic tree, the target tree species *Toona ciliata* (OM574630) and *Toona sinensis* (OM574631), belonging to the Meliaceae, clustered into a narrow branch with 100% support. This unifies with the results of traditional morphological taxonomy.

Since plants in the Sapindaceae are more susceptible to geographical location and their genetic variation, the evolutionary distance and genetic variation of plants within the Sapindaceae vary widely(Limin et al. 2014; Minghe 2012), and *Xanthoceras sorbifolium* (MK333231.1) and *Sapindus mukorossi* (MT806100.1), although *Xanthoceras sorbifolium* (MK333231.1) and *Sapindus mukorossi* (MT806100.1) are on the same branch line, the support between them is not high, only 56%.

Flora of China records that Meliaceae, Rutaceae, Anacardiaceae, Sapindaceae, and Nitrariacea are natural taxon. Taxonomists such as Rendle, Hutchinson, and others, who have organ morphological classification, have concluded that Meliaceae and Rutaceae are closely related, but for the classification of the degree of affinity between them, most of them are distinguished from plant physiology and morphology, less from the molecular level of genes(Ping, Yi, and Yingying 2003; Xueqin et al.). The establishment of the ML evolutionary tree provides a preliminary evolutionary relationship between Meliaceae and Rutaceae at the mitochondrial genome level, but there are limitations because the published mitochondrial genome sequences of plants are still quantitatively insufficient to represent the family level.

4. Conclusion

The completion of mitochondrial genome sequencing of *T. ciliata* and *T. sinensis* has enriched the mitochondrial genome library of *Toona*. and is important for investigating interspecific species relationships and researching the genetics and evolution of *Toona*.

The mitochondrial genomes are predominantly maternally inherited and do not originate in the recombinant genome, therefore, they may have dissimilar evolutionary mechanisms and might reflect different evolutionary information. Phylogenetic tree building also further illustrates that the simulation of mitochondrial genomic evolutionary tree outcomes is moderately compatible with the traditional classification. The mitochondrial genome can be acclaimed as a molecular marker for the investigative assessment of phylogenetic relationships among species and the genetic structure of populations.

Regarding *T. ciliata* and *T. sinensis*, it is of great value for the data on their energy metabolism, growth and development, and hybrid breeding.

Declarations

Plant Material:

I hereby declare that the plant material for this experiment was harvested in strict accordance with the relevant institutional, national and international guidelines and legislation, and that the material was used exclusively for scientific research purposes.

Availability of data and materials

The datasets generated during the current study are available in the [NCBI] repository, [Toona sinensis (GenBank:OM574631.1) and Toona ciliata (GenBanK:OM574630.1)] .

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Author Contributions:

Conceptualization, Youli Li and Min Gu; Data curation, Youli Li and Jiana Lin; Formal analysis, Min Gu and Jiana Lin; Funding

acquisition, Xingcui Xiao and Wei Zhou; Project administration, Xingcui Xiao; Supervision, Xingcui Xiao and Wei Zhou;

Validation, Jiana Lin; Writing – original draft, Youli Li and Min Gu.

All authors will be informed about each step of manuscript processing including submission, revision, revision reminder, etc.

Consent for publication

All authors consistently consent to publish the article.

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Figures

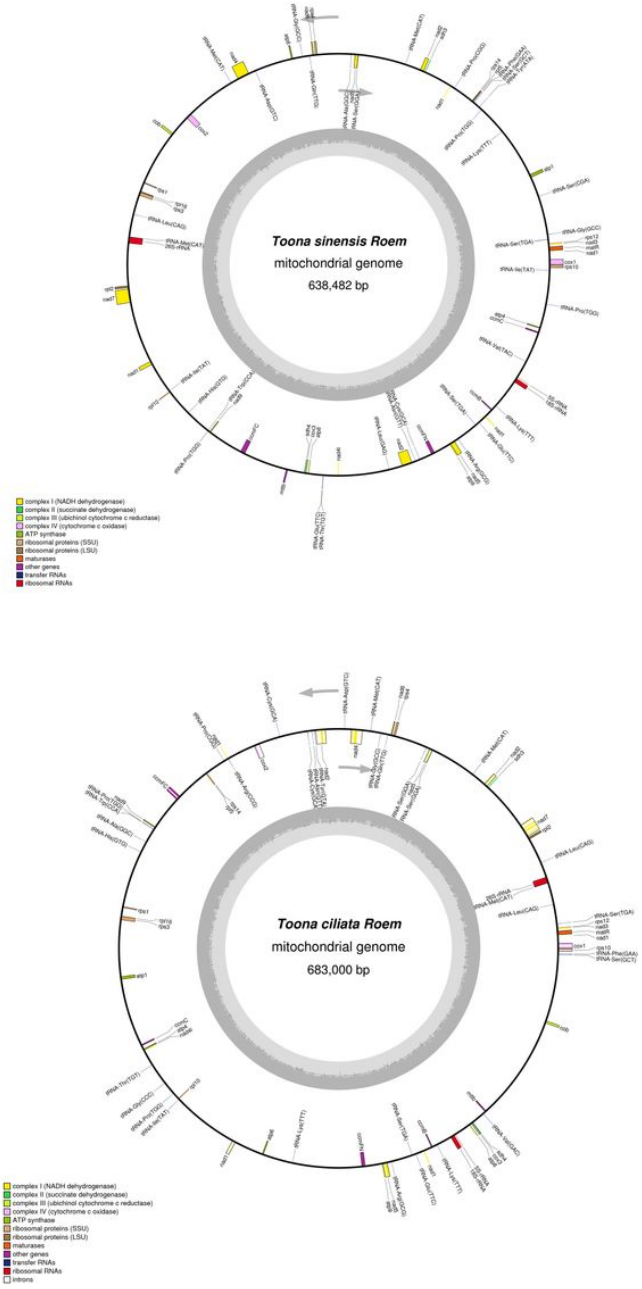

Figure 1

A map of the mitochondrial genome of the *Toona*. (A) *Toona sinensis*,(B) *Toona ciliate*.

Reverse transcription is indicated by genes outside of the circles, and clockwise transcription is indicated by genes inside the circles. The two IR regions are represented by the thick black line on the outside circle. The GC content is represented by the inner nucleus' dark gray graph.

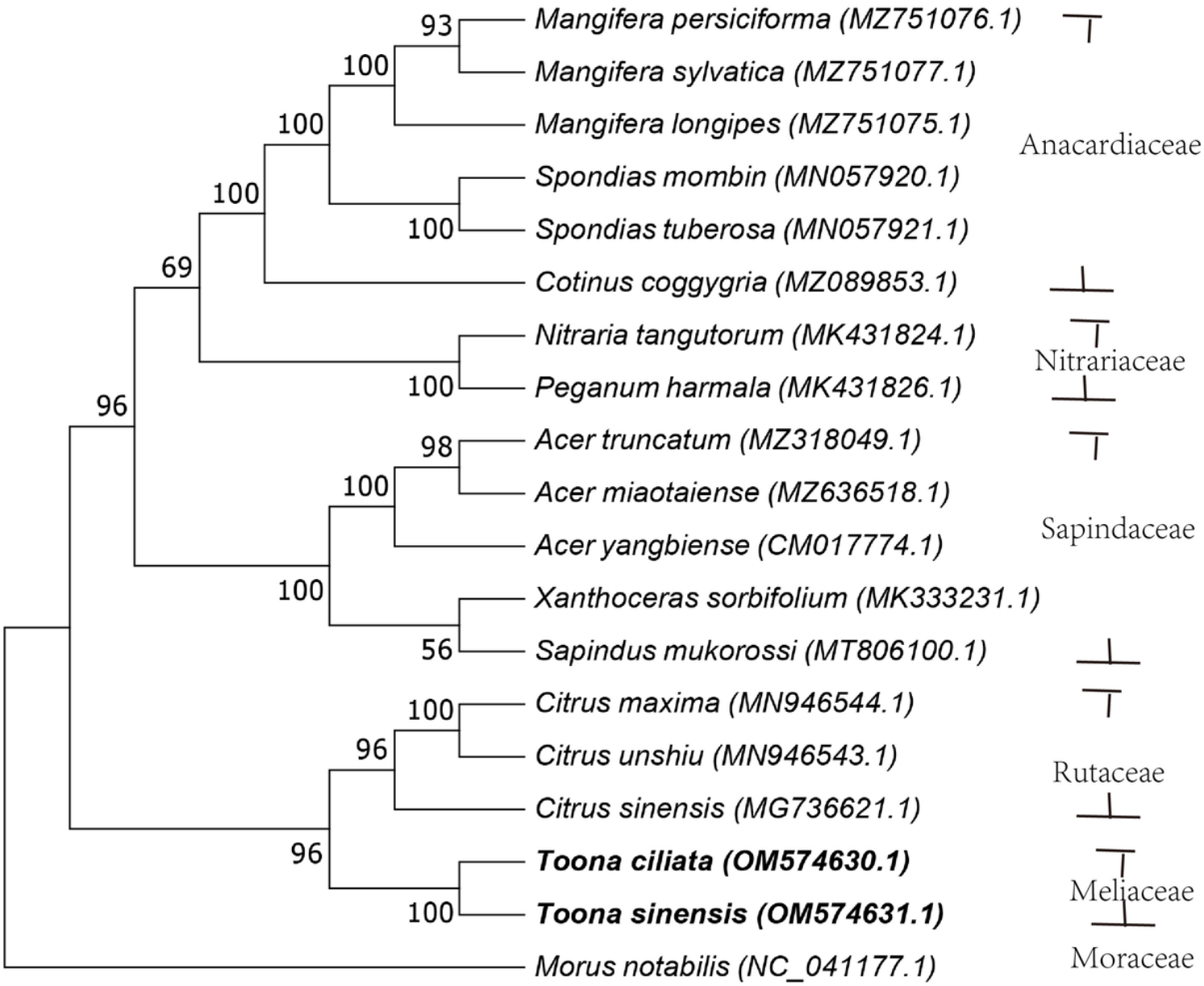

Figure 2

Phylogenetic trees constructed based on the ML method for 19 related plants.