Characterization of the complete mitochondrial genome of the new tea pest Mycterothrips gongshanensis (Insecta: Thysanoptera: Thripidae)

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Research Article

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Abstract

Background *Mycterothrips gongshanensis*, as a new tea pest, has spread wildly in Guizhou and Yunnan Province, China, and the damage can be comparable to that of the major tea pest *Dendrothrips minowai*. Though its bionomics have been documented, but lack molecular biology researches. The present study, complete mitochondrial genome of *M. gongshanensis* was firstly obtained and analysed.

Methods and Results We assembled the complete 15,154 bp circular mitochondrial genome of *M. gongshanensis* by the first-generation sequencing, which included 13 protein-coding genes (PCGs), 22 common tRNA genes, two repetitive tRNA genes (tRNA-Asn, and tRNA-Ile), two rRNA genes, and two control regions. The nucleotide composition is AT-biased (5.20%), with the respectively proportion as follow, 40.64% A, 12.67% C, 10.06% G and 36.62% T. The PCGs, with a whole length of 10,985 bp, the majority were initiated at typical start codons ATN (N, any nucleotide) and terminated with the typical stop codons TAA or TAG, except for atp8 using GTG and single T as start and stop codon, respectively. Additionally, the phylogenetic analysis indicated that *M. gongshanensis* was a sister group relationship with the genus *Thrips*, and the tribes Sericothripini and Dendrothripini were recovered as monophyletic group.

Conclusions Gene rearrangement consists in the mitochondrial genome of *M. gongshanensis*, which is most closely related to the *Thrips*. The results will contribute to the classification and rapid identifucations of Thripidae.

Introduction

Thrips, a group of the significant pests of tea trees, distribute worldwide [1–5]. Although more than 20 species of tea thrips have been reported in China [6–8], only two species, *Scirtothrips dorsalis* Hood [9] and *Dendrothrips minowai* Priesner [3, 10] had been being considered as the dangerous pests. *Mycterothrips gongshanensis* was introduced as a new species collected from Baoshan City of Yunnan Province and Liupanshui City of Guizhou Province, China in 2017, and suspected to be a potential pest in tea gardens [11]. During the past few years, the biological characteristics of *M. gongshanensis* has been tracked and the results revealed that it was a new tea pest and outbreak in over twenty counties and cities in Guizhou and Yunnan provinces [12–13]. Thus, *M. gongshanensis* has been a significant tea pest in southwest area of China and a rapid identification technique and population outbreak genetics for this pest are required.

Due to the small bodily form, concealment behavior and concurrence of similar species, accurate morphological identification of thrips was difficult for the non-taxonomist. However, molecular biological technique could be a nice way to solve this problem, and mitochondrial genome has been to a powerful molecular tool for rapid identification and genetic source determination [14]. In this study, the complete mitochondrial genome of *M. gongshanensis* was firstly obtained and analysed. The results will benefit the further studies of its distribution and phylogenetic relationships within Thripidae.

Materials And Methods

Sample collection and DNA extraction

Samples were collected from the tea garden (Huishui County, Guizhou Province, China; 26°8′56.97″N, 106°43′8.26″E) and preserved in absolute ethanol in a cryopreservation tube. Genomic DNA was extracted from adults of *M. gongshanensis* using the rapid extraction kit follow the instructions (Aidlab Biotechnologies Co., Ltd).

Mitochondrial Genome Sequencing And Annotation

The complete mitochondrial genome of *M. gongshanensis* was sequenced through the first generation sequencing. As a reference sequence, the complete mitogenomic sequence of *D. minowai* (GenBank accession No. MF582634) was used for primers design. The PCR reaction was performed using the LA Taq polymerase. PCR products were sequenced via the automatic sequencer (ABI 3730) [15]. The sequenced data was assembled and annotated using DNA star [16], then analyzed and adjusted manually to obtain the complete mitochondrial genome sequence.

Phylogenetic Analysis

To clarify the phylogenetic relationship within the family Thripidae, the phylogenetic tree was constructed based on the concatenated amino acid sequences of 13 PCGs. 12 Thripidae mitogenomes (including one newly sequenced mitogenome of *M. gongshanensis* and 11 downloaded sequences from GenBank) were used for the phylogenetic analysis. 11 species of Sericothripini and Dendrothripini were selected as ingroups and *Haplothrips aculeatus* of Haplothripini was selected as outgroup. The 13 PCGs were extracted using PhyloSuite 1.2.1 [17] and aligned using the MASCE [18] algorithm in PhyloSuite 1.2.1 with the invertebrate mitochondrial genetic code. Gblocks 0.91b [19]was employed to remove the gap and ambiguous sites with default settings. Bayesian inference (BI) analysis was constructed using MrBayes 3.2.6 [20] under the best partitioning scheme and models selected by PartitionFinder with the Akaike information criterion (AIC) [21]and greedy search algorithm. For the BI analysis, four simultaneous runs for 1,000,000 generations with a sampling frequency of 1000 generations, with the first 25% of samples were discarded as burn-in.

Results

The annotated mitogenomic sequence was submitted to GenBank with the accession number MZ913437. The complete mitogenome of *M. gongshanensis* was a closed loop with the length of 15,154 bp (Fig. 1). The figure was drawn using the CGView online server (http://cgview.ca/) with default parameters [22]. Differing from other Thrips [23–27], gene rearrangement occurred in the mitochondrial genome sequence of *M. gongshanensis*. Two repetitive tRNA genes (tRNA-Asn and tRNA-Ile) were

contained in this genome sequence besides the 37 typical mitochondrial genes (13 PCGs, 22 tRNAs, two rRNAs). Meanwhile, two A + T-rich regions (D-loop) were detected, which is similar to *Neohydatothrips samayunkur* [27], *Thrips imaginis* [25], and *T. palmi* [26], but less than *Frankliniella intonsa* [24] and *F. occidentalis* [25]. As seem as other Thrips [23, 25–26], 3 PCGs (nad4L, nad4 and nad5) and 3 tRNAs (tRNA-His, tRNA-Tyr and tRNA-Pro) were encoded on the light (L)-strand, while the rest genes were encoded on the heavy (H)-strands (Table 1). Within the complete mitochondrial genome of *M. gongshanensis*, 19 small non-coding portions (intergenic spacers) were found and a total of 18 bp overlaps were identified at 10 gene junctions, and two large non-coding regions were identified with 202 and 149 bp in length, respectively (Table 1). The nucleotide composition was AT-biased (5.20%), with the respectively proportion as follow: A = 40.64% (6159), C = 12.67% (1920), G = 10.06% (1525) and T = 36.62% (5550) (Table 2).

The PCGs, ranging from 196 bp (atp8) to 1689 bp (nad 5), with the total length of 10,985 bp (Table 2). All of the PCGs were initiated with typical start codons ATN (N, any nucleotide) and terminated with typical stop codons TAA or TAG, except for atp8 started with GTG and terminated with incomplete stop codon T. The AT skew of the PCGs nucleotide composition was 4.71%, slightly lower than that of the whole mitochondrial genome. And the A, T, G, C proportion were 39.99%, 36.39%, 10.00%, 13.62%, respectively (Table 2).

The tRNAs, ranging from 60 bp (Ile, Trp, Ser, Val) to 69 bp (Pro and Gln), with the total length of 1576 bp. All tRNAs have the conserved triple nucleotides recognizing corresponding codon. The nucleotide composition was AT-biased, with the ratio of 4.11% (Table 2).

The length of 16S rRNA and 12S rRNA gene are 1096 bp and 717 bp, respectively (Table 1). The AT skew of the rRNAs was 15.19%, higher than that of PCGs and tRANs obviously (Table 2).

As the A + T richest region, the proportion of control regions can reach 88.60%. Distinct to the genes, the AT skew of control regions was negative (Table 2).

Table 1
Annotations of the complete mitochondrial genome of Mycterothrips gongshanensis

Gene	Position		Size	Codon		Anticodon	Strand[a]	IGS[b] (bp)
	Start	End		Start	Stop			
nad4	1	1320	1320	ATT	TAA		L	0
nad4L	1314	1580	267	ATG	TAG		L	-7
tRNA-Cys	1652	1715	64			GCA	H	71
nad6	1721	2215	495	ATC	TAA		H	5
tRNA-Val	2241	2300	60			TAC	H	25
16S rRNA	2301	3396	1096				H	0
tRNA-Ser	3397	3462	66			TGA	H	0
cox1	3477	5018	1542	ATG	TAA		H	14
nad3	5018	5371	354	ATG	TAA		H	-1
tRNA-Leu	5375	5440	66			TAA	H	3
cox2	5441	6103	663	ATA	TAA		H	0
tRNA-Asp	6114	6178	65			GTC	H	10
tRNA-Arg	6179	6243	65			TCG	H	0
tRNA-Gly	6244	6309	66			TCC	H	0
tRNA-Lys	6316	6378	63			TTT	H	6
cox3	6411	7205	795	ATA	TAA		H	32
tRNA-Asn	7294	7360	67			GTT	H	88
tRNA-Ile	7378	7444	67			GAT	H	17
CR[c]1	7445	7646	202					0
tRNA-Trp	7647	7713	67			TCA	H	0
nad1	7714	8640	927	ATA	TAA		H	0
tRNA-Met	8637	8701	65			CAT	H	-4
tRNA-Ala	8702	8766	65			TGC	H	0
tRNA-Phe	8767	8833	67			GAA	H	0
12S rRNA	8834	9550	717				H	0
atp8	9551	9746	196	GTG	T		H	0

Gene	Position		Size	Codon		Anticodon	Strand[a]	IGS[b] (bp)
	Start	End		Start	Stop			
atp6	9764	10417	654	ATT	TAA		H	17
tRNA-Ser	10431	10490	60			TCT	H	13
tRNA-Leu	10491	10557	67			TAG	H	0
tRNA-Thr	10565	10630	66			TGT	H	7
tRNA-Pro	10634	10702	69			TGG	L	3
tRNA-Gln	10714	10782	69			TTG	H	11
tRNA-Glu	10806	10871	66			TTC	H	23
cytb	10917	12026	1110	ATA	TAG		H	45
tRNA-Tyr	12024	12090	67			GTA	L	-3
nad2	12123	13103	981	ATT	TAA		H	32
tRNA-Asn-2	13104	13170	67			GTT	H	0
tRNA-Ile-2	13188	13254	67			GAT	H	17
CR[c]2	13255	13403	149					0
nad5	13404	15092	1689	ATT	TAA		L	0
tRNA-His	15090	15154	65			GTG	L	-3

[a]H heavy strand, L light strand; [b]IGS denotes the length of the intergenic spacer region, for which negative numbers indicate nucleotide overlapping between adjacent genes; [c]CR control region.

Table 2
Nucleotide composition of the complete mitochondrial genome of Mycterothrips gongshanensis

Gene sequnences	Size(bp)	A%	G%	T%	C%	AT%	GC%	AT skew(%)	GC skew(%)
Whole genome	15154	40.64	10.06	36.62	12.67	77.27	22.73	5.20	-11.48
Protein coding genes	10985	39.99	10.00	36.39	13.62	76.38	23.62	4.71	-15.33
tRNA genes	1576	41.18	10.34	38.07	10.41	79.25	20.75	3.92	-0.34
rRNA genes	1813	44.73	11.69	32.93	10.65	77.66	22.34	15.19	4.66
Control regions	351	37.32	5.13	51.28	6.27	88.60	11.40	-15.76	10.00

The phylogenetic analysis indicated that Sericothripini and Dendrothripini were monophyletic with most nodes received high support (Bayesian posterior probability (PP) ≥ 0.96). Within the tribe of Sericothripini, the phylogenetic relationship indicated that *M. gongshanensis* was sister group relationship with the genus *Thrips*, and the genus *Frankliniella* was recovered as a sister group with the clade that *Scirtothrips dorsalis* and *Neohydatothrips samayunkur* formed (Fig. 2). Compared with the previous studies based on the mitochondrial genome of Thripdae [25–28], more mitochondrial genome sequences were selected to construct the phylogenetic tree in this study. While the generic relationship within Thripdae remained questionable, which was not consistent with previous studies. To clarify the phylogenetic and evolutionary relationships within Thripdae, further studies with more taxon samples are much needed.

Conclusions

For the first time, the character of molecular structure and nucleotide composition of the complete mitochondrial genome of *M. gongshanensis* were analyzed in this study. The results showed that the gene rearrangement occurred in the mitochondrial genome sequence, two repetitive tRNA genes (tRNA-Asn and tRNA-Ile) were detected. Meanwhile, phylogenetic tree of 12 Thripidae species provides evidence that *M. gongshanensis* was closest to *Thrips*. These data will contribute to the rapid identification and population genetics of *M. gongshanensis* and evolutionary analysis for Thripidae.

Declarations

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Competing Interests No potential conflict of interest was declared by the coauthor.

Author contributions Chaojin Dao and Yufeng Zhou designed and conceived this study. Shuai Li and Wen Yang collected the *M. gongshanensis* samples. Shuai Li and Zehong Meng performed the data statistics. Shaui Li and Jinfeng Zhang constrcted and analysised the Phylogenetic tree. Shuai Li wrote the manuscript, improved and polished by all the athors.

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Data availability The sequence data generated in this study were openly available in GenBank (https://www.ncbi.nlm.nih.gov).

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Figures

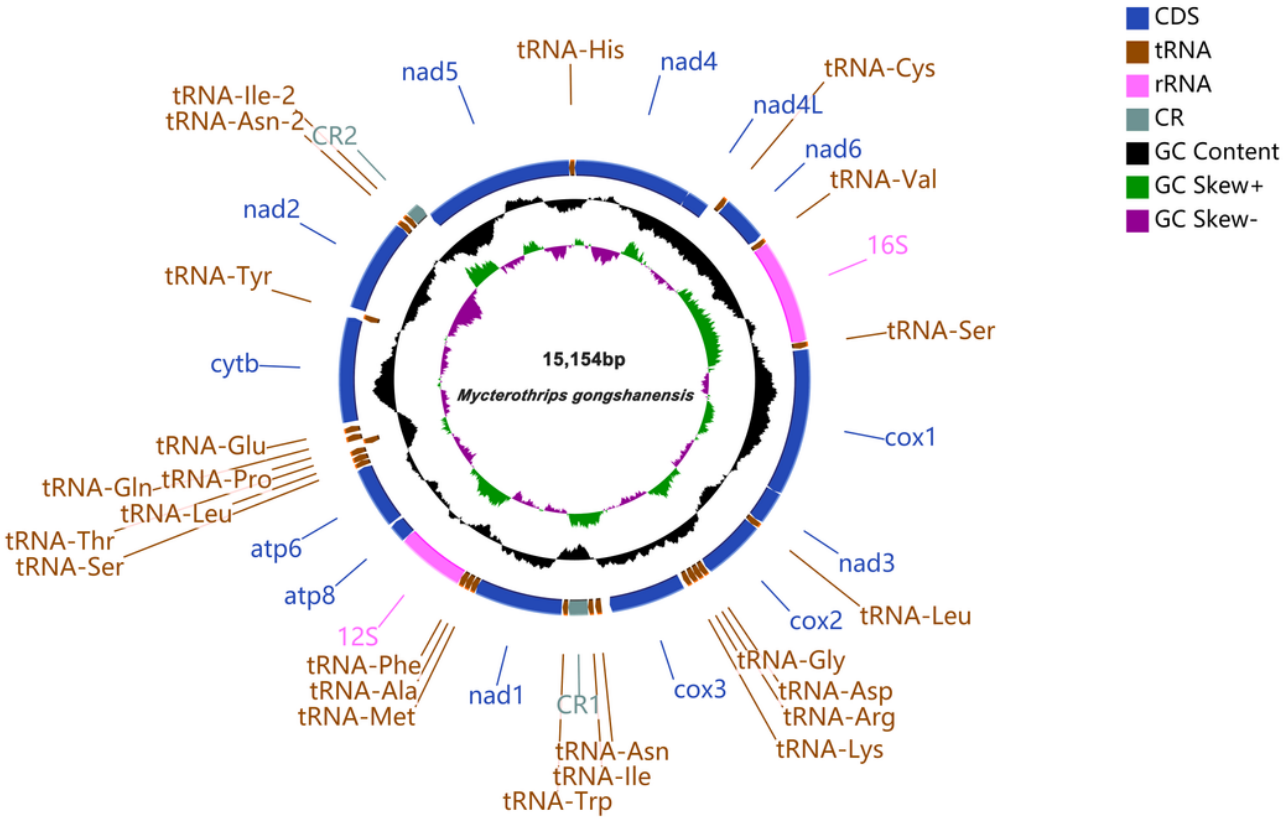

Figure 1

Physical map of the complete mitochondrial genome of Mycterothrips gongshanensis. Genes illustrated on the outside of the main circle are encoded on the heavy (H) strand; genes on the inside of the circle are encoded on the light (L) strand. The 13 PCGs are labeled in bule, 22 tRNA genes are labeled in brown, and LrRNA and SrRNA genes are labeled in pink. The GC content is plotted using a black sliding window, as the deviation from the average GC content of the entire sequence. The GC-skew is plotted using a colored sliding window (green and orchid color), as the deviation from the average GC skew of the entire sequence. The figure was drawn using the CGView online server (http://stothard.afns.ualberta.ca/cgview_server/) with default parameters.

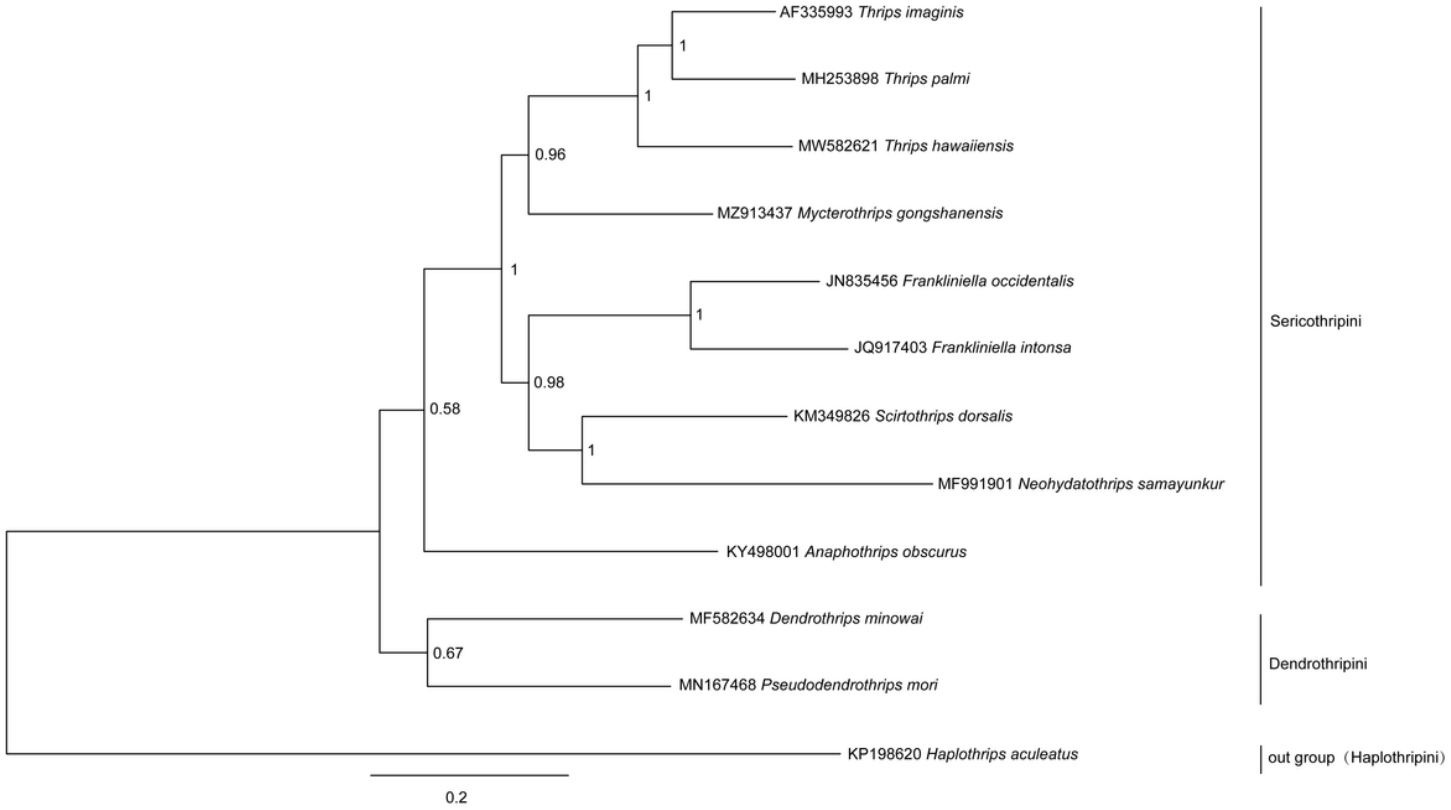

Figure 2

Phylogenetic tree of 12 species of Thripidae based on the amino acid sequences of 13 PCGs (constructed by MrBayes 3.2.6 under the best partitioning scheme and models selected by PartitionFinder 2, run for 1,000,000 generations with a sampling frequency of 1000 generations).

Supplementary Files

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- Adultsandtheleafdamage.jpg