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Characterization of the complete mitochondrial genome and phylogenetic analysis of bean thrips *Megalurothrips usitatus* (Bagnall, 1913) (Thysanoptera: thripidae)

Lin Xing-Ming, Li Pan, Cai Du-Cheng and Li Jin-Hua

Key Laboratory of Green Prevention and Control of Tropical Plant Diseases and Pests, Ministry of Education/School of Plant Protection, Hainan University, Haikou, P.R. China

ABSTRACT

Megalurothrips usitatus (Bagnall, 1913) (Thysanoptera: Thripidae) is a widely distributed pest in Asia that primarily affects the production of snap beans and cowpea. The complete mitochondrial genome of *Megalurothrips usitatus* has been sequenced and annotated in this study, which is 17,209 bp long and contains 13 protein-coding genes (PCGs), two rRNAs, and 22 tRNA genes. Most of the protein-coding genes (PCGs) start with ATG except *ND4* using TTG. Meanwhile, eight PCGs stop with TAA, four PCGs have an incomplete stop codon, and the gene *Cytb* ends with TAG. Phylogenetic analysis showed that *M. usitatus* is closely related to *Frankliniella intonsa* and *F. occidentalis*, providing a basis for the study of the mitochondrial evolution of Thripinae.

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Mitogenome; protein-coding genes; transfer RNA; ribosomal RNA; phylogenetic position

1. Introduction

Megalurothrips usitatus (Bagnall, 1913) (Thysanoptera: Thripidae) is the most significant pest on legumes in Asia (Mound and Kibby 1998; Tang et al. 2015), causing severe damage to cowpeas and snap beans in southern regions of China. The female *M. usitatus* is approximately 1.6 mm size and has a brown coloration (as shown in Figure 1). It possesses 8-sectioned antennae, and has compound and monocular eyes. The mesosternal furca has a spinula, while the metafurca does not. The narrow wings of the female have fine tassels peripherally. They are colorless near the base 1/4 of the forewing and near the tip, while the middle and tip are brown (Palmer, 1987). *M. usitatus* feeds on young tissues, including growing points, young flowers, and flower buds, causing leaf shrinkage, deformation, and the loss of flowers and pods, ultimately affecting yield (Huang et al. 2018; Liu et al. 2020). However, identifying this group based on morphological features alone is difficult due to their small size and species abundance, making it necessary to use molecular data accurately for exploring the taxonomic status of each species within this subfamily (Qiu et al. 2014). To enhance the comprehension of molecular evolution and taxonomic classification of thrips, we sequenced and characterized the mitochondrial genome of *M. usitatus*.

2. Materials and methods

2.1. Sample collection

M. usitatus was obtained from Haikou (E110°11′38″, N20°02′45″), Hainan Province, China, in 2020. The specimens were photographed, morphologically identified, stored in absolute ethanol, and placed in a freezer at −20 °C. The specimen was deposited at School of Plant Protection, Hainan University (https://hd.hainanu.edu.cn/zhiwu/xwzx/jx_kyfwpt/bbg.htm, contact person: Jinhua Li; email: lijinhua@hainanu.edu.cn) under the voucher number Mus-hn-L1.

Figure 1. The female of *Megalurothrips usitatus*. Photographed by Xingming Lin.

CONTACT Jin-Hua Li lijinhua@hainanu.edu.cn School of Plant Protection, Hainan University, Haikou, P.R. China.
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2.2. Methods

Total genomic DNA was extracted from the 3rd-instar larvae and then sequenced on Illumina NovaSeq 6000 platform. The mitochondrial genome sequence was assembled using NOVAPlasty (2.4 version) and annotated with Geseq (https://chlor-obox.mpimp-golm.mpg.de/geseq.html) (Tillich et al. 2017) and MITOS (http://mitos2.bioinf.uni-leipzig.de/index.py) (Bernt et al. 2013), and then illustrated by OGDRAW online software (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html). To validate the reliability of the genome, a comparison was made with mitogenomes of other Thysanoptera species, and two of them, *Aeolothrips xinjiangensis* and *Franklinothrips vespiformis*, were used as outgroups. The complete mitochondrial genome sequences of these 13 insect species were downloaded from NCBI database. Nucleotide sequences of all protein-coding genes and ribosomal genes were individually aligned and concatenated using Geneious Prime 2021 (Kearse et al. 2012). MrModeltest version 2.4 (Darriba et al. 2020) was used to select the best-fit model. Maximum likelihood phylogenies were inferred using IQTREE (Nguyen et al. 2015) under the GTR + I + G model for 1000 bootstraps. Bayesian Inference phylogenies were inferred using MrBayes 3.2.1 (Ronquist et al. 2012) based on Markov Chain Monte Carlo (one cold and three hot chains) chains of 2,000,000 with 25% burn-in, and sampling was done every 100 generation. The final average standard deviation of split frequencies was 0.0007. The evolutionary tree is visualized in itol (https://itol.embl.de/) (Letunic and Bork 2007).

3. Results

The coverage-depth map was provided, and the average depth was 770 x (Supplementary materials, Figure S1). The complete

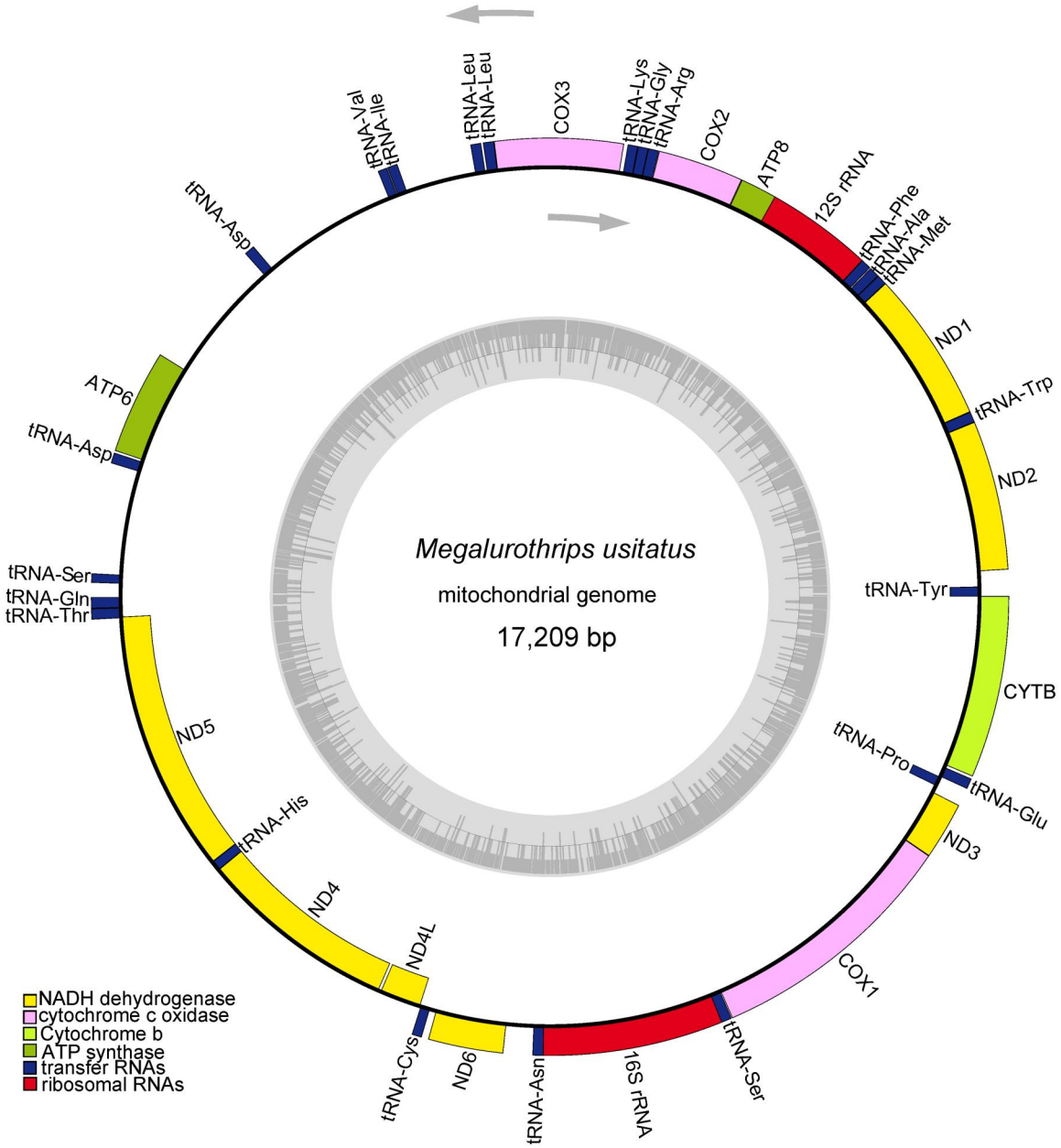

Figure 2. The mitochondrial genome map of *Megalurothrips usitatus*. Genes are shown outside and inside the outer circle are transcribed counterclockwise and clockwise, respectively. GC and AT contents across the mitochondrial genome is shown with dark and light shading, respectively, inside the inner circle.

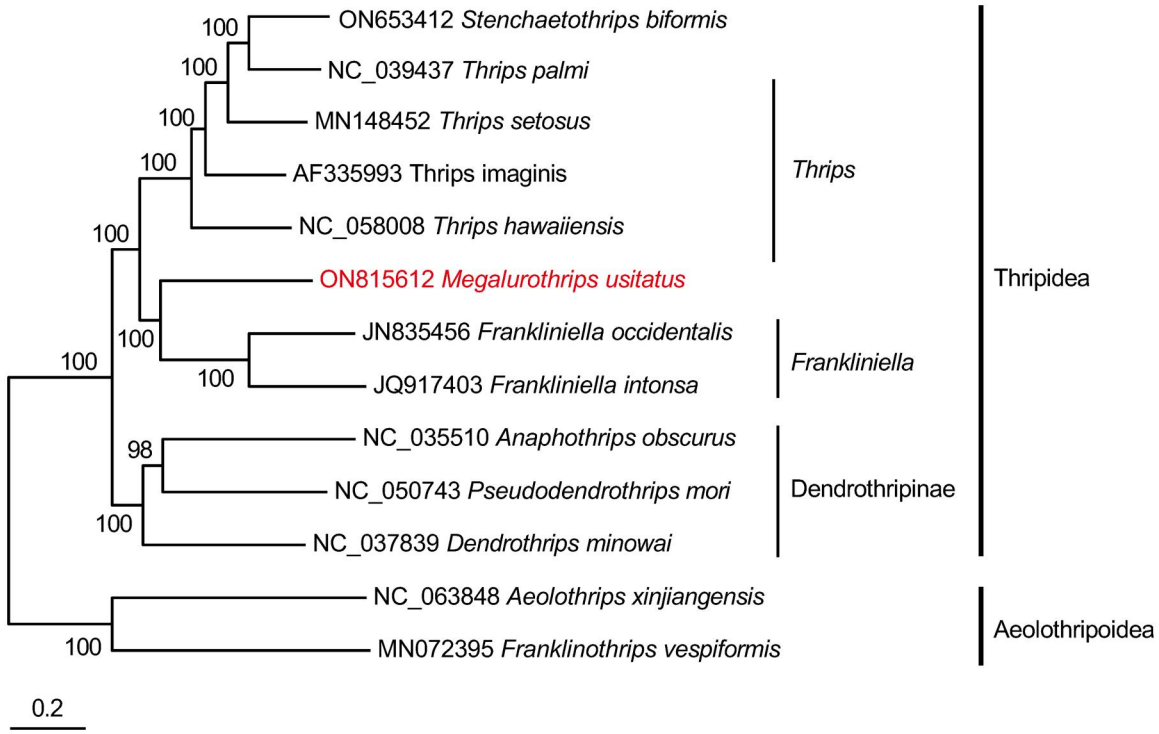

Figure 3. Phylogenetic tree of 13 insect species, including *Megalurothrips usitatus* based on the nucleotide dataset of the 13 mitochondrial protein-coding genes and ribosomal genes. 'GTR + I + G' was used as the best-fit nucleotide substitution model. Bayesian Inference phylogenies were inferred using Markov Chain Monte Carlo (one cold and three hot chains) chains of 2,000,000 with 25% burn-in, and sampling was done every 100 generation. The posterior probabilities are indicated above the nodes. The GenBank accession no. of sequences used in the study are *stenchaetothrips biformis* ON653412 (Hu et al. 2023); *Thrips palmi* NC_039437 (Chakraborty et al. 2018); *Thrips setosus* MN148452; *Thrips imaginis* AF335993 (Shao and Barker 2003); *Thrips hawaiiensis* NC_058008; *Megalurothrips usitatus* ON815612 (this study); *Frankliniella occidentalis* JN835456 (Yan et al. 2012); *Frankliniella intonsa* JQ917403 (Yan et al. 2014); *dendrothrips minowai* NC_037839; *anaphothrips obscurus* NC_035510 (Liu et al. 2017); *pseudodendrothrips mori* NC_050743; *Aeolothrips xinjiangensis* NC_063848; *Franklinothrips vespiformis* MN072395 (Tyagi et al. 2020).

mitochondrial genome was 17,209 bp long, and the total length of all 13 protein-coding genes (*ND1-ND6*, *ND4L*, *COX1-COX3*, *ATP6*, *ATP8*, *Cytb*), accounting for 62.14% of the whole genome sequence. In addition to these 13 protein-coding genes, the genome contains two rRNAs and 22 tRNA genes (Figure 2). The tRNA genes are between 56 and 68 bp in length. The total nucleotide composition of the genome is 42.02% A, 11.98% C, 10.20% G, and 35.80% T. The A + T content (77.83%) is significantly higher than that of G + C (22.17%), consistent with most insect mitochondrial genomes (Tyagi et al. 2020). Most of the protein-coding genes start with ATN including ATA for *ND1*, *ND5*, *COX2*, *Cytb*, *ATP8*, and ATT for *ND2*, *ND3*, *COX3*, *ATP6* and ATG for *ND4L*, *ND6*, *COX1*, *ATP8*, except *ND*4 which uses TTG. Meanwhile, eight PCGs have TAA as the stop codon, *Cytb* ends with the unfrequent stop codon TAG, while *ND1*, *ND2*, *ND4* and *ATP8* have an incomplete stop codon T-. Gene editing can be employed to ensure the proper functioning of the stop codon in these cases. The incomplete stop codon T is commonly reported and could produce functional stop codons in polycistronic transcription cleavage and polyadenylation mechanisms (Boore 2001). *S-rRNA* and *L-rRNA* have a 730 bp and 1162 bp in length for each. Phylogenetic analysis indicated that *M. usitatus* belongs to the tribe Thripidae, and is closer to its sister clade *Frankliniella* (Figure 3).

4. Discussion and conclusion

The mitochondrial genome of *M. usitatus* was 17,209 bp in length, and it contained 13 protein-coding genes (PCGs), 22 tRNAs and 2 rRNA genes. The analysis showed that the genomic structure of *M. usitatus* is similar to most bilateral animals previously studied (Yan et al. 2014). Phylogenetic analysis revealed the phylogenetic position of *M. usitatus* and indicated that *M. usitatus* belongs to the tribe Thripidae, a sister clade to the species of *Frankliniella*. Additionally, our study yields significant insights into the mitogenome of *M. usitatus*, which will facilitate further research on evolution, germplasm identification, and molecular markers development. Consequently, it is thought that the phylogenetic analysis conducted in this study can distinguish species of Thysanoptera by utilizing mitochondrial gene sequences.

Author contributions

J-H L planned and designed the research. P L collected the insect materials, X-M L performed experiments, D-C C and X-M L analyzed the data. X-M L and P L wrote the manuscript.

Ethics approval and consent to participate

The material used in this study is widely distributed in southern regions of China and does not belong to the IUCN Red List. No specific permits were required for animal collection. The study did not require ethical approval or consent, as no endangered or protected animal species were involved.

Disclosure statement

All authors have read and approved the final manuscript. No conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession no. ON815612. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA935568, SRP423475 and SAMN33321779, respectively.

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