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Unravelling the complete mitochondrial genomes of *Thrips tabaci* Lindeman and *Thrips parvispinus* Karny (Thysanoptera: Thripidae) and their phylogenetic implications

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Onion (*Allium cepa* Linnaeus) is an important vegetable crop valued for its nutritional properties and economics worldwide. Onion cultivation faces serious threats from pests and diseases, particularly onion thrips (*Thrips tabaci*), which cause substantial yield losses. Recently, Black thrips (*Thrips parvispinus*), an invasive key pest of chili, have been reported to cause severe damage in onion crop and is likely to devastate the onion cultivation in near future. Therefore, this study was conducted to address the knowledge gap concerning the genetic basis and evolutionary history of *T. tabaci* and *T. parvispinus* through sequencing of their mitochondrial genomes. *T. tabaci* and *T. parvispinus* were collected from different locations in Maharashtra, India, and reared in the laboratory. The mitochondrial genomes of *T. tabaci* and *T. parvispinus* were sequenced to a length of 15,277 and 15,285 bp, respectively. Both genomes exhibited similar gene organization with regard to thirteen protein-coding genes and two *rRNA* genes. *T. tabaci* contained 19 *tRNA* genes whereas *T. parvispinus* contained 18 *tRNA* genes. The evolutionary positions of *T. tabaci* and *T. parvispinus* within the Thysanoptera order were elucidated through phylogenetic analysis of the mitogenomes of 15 thrips species. These findings provide crucial insights into the genetic makeup and evolutionary dynamics of both the thrips species, thereby aiding the development of novel and sustainable pest management strategies to mitigate their impacts on crops in the changing climate scenario.

Introduction

Onion (*Allium cepa* L.) is widely acknowledged as an important vegetable crop worldwide, extensively grown and consumed for its culinary and nutritional value, and plays a vital role in the economies of many countries (1). Onion cultivation is particularly widespread in India, which is one of the world's leading producer and exporter (2). However, onion production faces numerous challenges due to the prevalence of various pests and diseases, which lead to substantial yield losses (3). Globally, onion growers are concerned about onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (4, 5). In India, this pest is considered of national significance, causing 30% to 40% yield loss in onion crops (6, 7). Thrips infestation not only weakens the plants but also makes them susceptible to secondary infections and diseases, which exacerbates economic impacts on farmers (8, 9). These pests are known vectors for various plant viruses, including the iris yellow spot virus (8, 10), and they also transmit diseases like Stemphylium leaf blight and bacterial leaf blight (3, 11–13). Virus-vector relationship have been well documented in the case of thrips; however, their interaction with fungal pathogens remains largely unexplored (Saini et al., 2024). Moreover, thrips usually exhibit genetic heterogeneity, which might be due to the spatial variation in insecticide efficacy (14). Based on mitochondrial DNA sequences, *T. tabaci* has been classified into three biotypes: one associated with tobacco and two associated with leek (L1 and L2) (15). Also, due to the anticipated increase in temperature, the lifecycle of *T. tabaci* is likely to shorten, leading to multiple generations within a single crop season (16). Currently, onion growers rely on chemical pesticides to manage these pests, but it often seems futile due to their overlapping generations, concealed feeding behavior, and growing insecticide resistance (17, 18).

Similarly, *Thrips parvispinus* (Southeast Asian thrips or black thrips), are known to infest a variety of host plants, including vegetables, ornamentals, and field crops. Recently, *T. parvispinus* has been found infesting onion crops (19), which could pose a substantial threat in the near future (20). The inclusion of *T. parvispinus* in the list of onion pests reveals a potential gap in our understanding of thrips species interactions and their impact on onions. A comprehensive understanding of the biology, genetics, and evolutionary relationships of thrips is required to tackle these pests in onion and to devise appropriate management strategies. Recent advancements in molecular biology have enabled researchers to explore the genetic makeup of various organisms, providing insight into their evolutionary histories and ultimately helping in devising novel pest management strategies.

The increasing interest in mitochondrial genomes for phylogenetic studies has led to a surge in published mitogenome sequences, particularly amongst insects. Although the complete mitochondrial genomes of several thrips species, such as *Thrips imagines* (21), *Frankliniella occidentalis* (22), *Frankliniella intonsa* (23), *Scirtothrips dorsalis* (24), *Anaphothrips obscurus* (25), *Thrips palmi* (26), *Dendrothrips minowai* (27), *Thrips hawaiiensis* (28), *Thrips parvispinus* (29) and *Aptinothrips stylifer* (30), have been sequenced, comprehensive research specifically focusing on onion

thrips is unexplored. Insect mitogenomes are small, circular, and consist of 37 genes: 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), and 22 transfer RNA genes (tRNAs), along with a large A+T-rich control region (CR) that regulates transcription and replication (31, 32). Their maternal inheritance, conserved gene content, and rapid evolutionary rate make mitogenomes valuable molecular markers for evolutionary research (33). The significant variation in mitochondrial genome organization observed within the subfamily Thripinae is highly unusual and contrasts with patterns seen in most other animals. The reasons behind the rapid evolution of mitochondrial genomes in Thripinae, as well as the evolutionary dynamics of mitochondrial genomes in other thrips, remain to be explored (25). In this context, the present study on the complete mitogenome of *T. tabaci* and its phylogenetic implications is of great significance. Furthermore, comparative studies of different thrips mitogenomes will help in elucidating the evolutionary patterns and population dynamics within the Thysanoptera order. Therefore, the study aims to address the knowledge gap by presenting the complete mitochondrial genome sequences of *T. tabaci* and *T. parvispinus*. This will offer valuable insights into their evolutionary relationships, population structure, and genetic diversity, ultimately aiding in devising an effective pest management strategy.

Materials and methods

Sample collection

Adults of onion thrips were collected from onion plants and initially reared in the laboratory on French beans at the ICAR-Directorate of Onion and Garlic Research (ICAR-DOGR) in Pune, Maharashtra, India (latitude: 18.84°N, longitude: 73.88°E, 616.29 meters above sea level). After completing their life cycle, adults emerged from individual eggs, were collected and used for further analysis. Meanwhile, Black thrips from chili plants in a farmer's field (latitude: 18.87°N, longitude: 74.05°E, elevation: 667.84 meters) were collected and preserved in 99% ethanol for further analysis. The species identity of these specimens was confirmed through DNA barcoding of the *COX-1* gene, and their sequence information has been submitted in the NCBI GeneBank database with accession numbers PP980527 and PP982736 for *T. tabaci* and *T. parvispinus* respectively.

Sample preparation and DNA isolation

Single adults of *T. tabaci* and *T. parvispinus* were macerated in liquid nitrogen, and total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Germany). The integrity of isolated total DNA was visualized on 1% agarose gel and further quantified using a nanodrop (Bio-Rad, Hercules, California, USA). Mitochondrial DNA was then synthesized from the total DNA using the REPLI-g Mitochondrial DNA Kit (QIAGEN, Germany).

Sequence assembly, annotation, and analysis

The genome library was constructed with the QIASeq FX DNA kit (QIAGEN, Germany), and sequencing was performed on an Illumina NextSeq 2000 platform using 300-cycle paired-end chemistry, generating primary FASTQ data. These FASTQ files were assessed for total bases, read counts, GC%, Q30, and uncertain base percentages. Reads of high quality were obtained by eliminating adaptor contamination, ambiguous reads, and junk sequences using the fastp tool (v0.12.4) (34). Subsequently, BWA MEM (v0.7.17) was used to align the cleaned reads to the reference sequence (35). Protein-coding and RNA genes were identified from the consensus sequence with SAM tools' mpileup (36). A *de novo* assembly using a de Bruijn graph approach was performed to construct longer DNA contigs, and the mitogenome was assembled using the MEGAHIT tool (37) as part of the MitoZ package (38). The quality of assembly was evaluated using the Quast tool (39).

Gene annotation for the mitogenome was conducted with the Prokka annotation tool (40) on the Proksee web server (https://proksee.ca/) (41), producing a circular genome map, GC concentration, and GC skew. For tRNA gene structure prediction and mitogenome assessment, MITOS2 (42) at the Galaxy Europe Web Server (https://usegalaxy.eu/) was used.

The relative synonymous codon usage (RSCU), codon usage, and base composition (A+T contents) of the PCGs were analyzed using MEGA Software (v11.0.13) (43). The GC skewness was computed using the formula GC skew = (G-C)/(G+C); whereas the formula AT skew = (A-T)/(A+T) was used for determining the AT skewness (44). Intergenic spacers and gene overlaps were manually determined. The complete mitochondrial genomes of *T. tabaci* and *T. parvispinus* were submitted to the NCBI genome database under accession numbers PQ197393 and PQ197392, respectively.

Phylogenetic analysis

The evolutionary studies related to *T. tabaci* and *T. parvispinus* with the metagenome of 14 other thrips species were analyzed using the NCBI retrieved sequence information. The damsel bug, *Alloeorhynchus bakeri*, was used as an outgroup species. The nucleotide sequences of 13 protein coding genes of 16 thrips species and one outgroup species were aligned individually using MAFFT 7, the Database of Aligned Structure Homologue (DASH) was utilized to incorporate homologous structures based on amino acid codons (45). Followed by removal of the ambiguously aligned sites, the aligned amino acid sequences were then converted to the nucleic acid sequence. For phylogenetic analysis the sequences of 13 PCGs were concatenated in following order: nad5, nad4, nad4L, nad6, cox1, nad3, cox2, cox3, atp6, atp8, nad1, nad2 and cytB in a single sequence of each species. All the 17 sequences were aligned using CLustalW tool (46). The phylogenetic tree was constructed using the Maximum Likelihood method in MEGA 11 software (43), employing the general time reversible model with gamma distribution (GTR+G) and bootstrap values from 500 iterations.

Results

Structure and composition of mitogenome

The circular genome of *T. tabaci* is 15,277 bp long (Figure 1A), comprising 34 sequence elements (such as 13 PCGs, 19 tRNAs, and 2 rRNA-coding genes). Strand localization analysis revealed 19 genes located on the H-strand (+), whereas 15 genes on the L-strand (−) (Table 1). Gene length in the *T. tabaci* mitochondrion was 400 bp on average, with minimum and maximum lengths of 57 bp (trnS1) and 1695 bp (nad5), respectively. Base compositions of the complete mitochondrial genome of *T. tabaci* were 41.3%, 34.8%, 12%, and 11.9% for A, T, G, and C nucleotides. The 13 PCGs and two rRNAs had AT contents ranging from 69.80% to 82.50%, whereas the overall mitogenome had 76.10%. In contrast, the GC content varied from 17.50% to 30.20% for the 13 PCGs and 2 rRNAs, whereas the overall mitogenome had 23.90%.

The AT skewness for the 13 PCGs ranged from 0.426 to 0.033, higher than that of the rRNA genes, rrnS (-0.193) and rrnL (0.180) (Table 2). The 1560 bp of intergenic nucleotides were spread across 23 locations, with individual spacer lengths ranging from 2 to 451 bp. The longest intergenic spacer (451 bp) was found between the trnD and trnR genes. There were overlaps between nine genes, whose lengths varied from 1 to 54 bp. The atp8 and rrnS genes had the lengthiest overlap, 54 bp, as shown in Table 1 and Figure 1A.

Likewise, the circular mitogenome of *T. parvispinus* is 15,285 bp, comprising 13 PCGs, 18 tRNA-coding genes, and 2 rRNA-coding genes, 28 of which are located on the H-strand (+) the remaining 5 on the L-strand (−). Gene length in the *T. parvispinus* mitochondrion was 428 bp on average, with minimum and maximum lengths of 61 bp (trnS1) and 1695 bp (nad5), respectively (Figure 1B, Table 3). Our result was in accordance with Pakrashi et al. (29), revealing a mitogenome length of 15,067 bp. The mitogenome of *T. parvispinus* had base compositions of 43.3% A, 30% T, 10% G, and 11.7% C, with an overall AT content of 73.3% and GC content of 21.7%. The AT content across the 13 PCGs and 2 rRNAs ranged from 72.8% to 84%, while the GC content varied from 17% to 27.2%. AT skewness was higher in the PCGs (0.064 to 0.410) compared to the rRNAs (0.161 for rrnS and 0.187 for rrnL) (Table 4). We identified 1,039 bp of intergenic nucleotides across 24 locations, with the longest spacer (347 bp) between the trnE and trnP genes. Seven genes had overlaps, with the largest (35 bp) between the COX2 and ND4 genes (Table 3, Figure 1B).

Protein-coding genes and codon usage bias

The mitochondrial genomes of *T. tabaci* and *T. parvispinus* exhibit distinct characteristics in their PCGs. The total lengths of the 13 PCGs were 10,584 bp for T. *tabaci* and 11,055 bp for *T. parvispinus*. In *T. tabaci*, the coding sequences ranged from 126 bp (atp8) to 1,542 bp (cox1), with genes such as cox1, cox2, cox3, nad3, and nad6 located on the H-strand, while others like nad1, nad2, nad4, nad4L, nad5, atp6, atp8, and cytB were on the L-strand. Conversely,
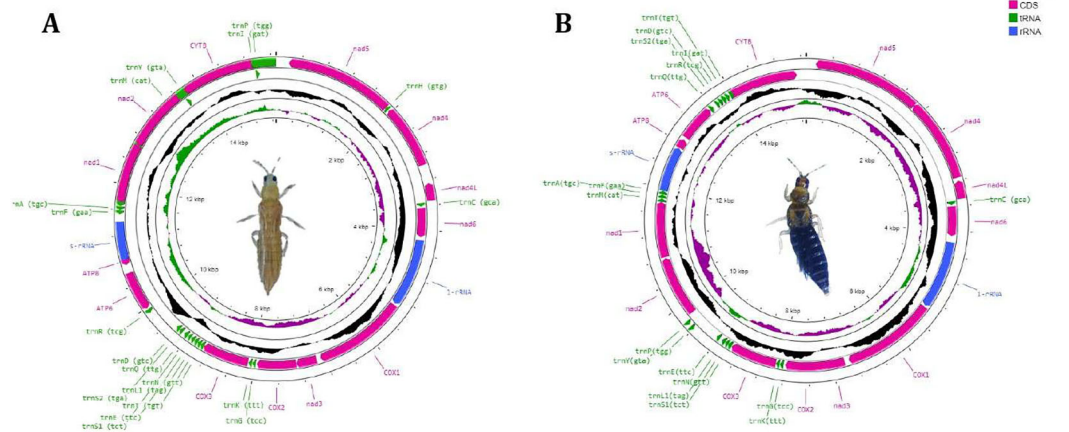
FIGURE 1
Circular genome map of **(A)** *T. tabaci* and **(B)** *T. parvispinus* showing mitogenome sequence features.

TABLE 1 Summary of *Thrips tabaci* mitochondrial genome.

Gene Name	Full Name and Function	Gene Type	Position		Length (bp)	Strand	Intergenic Space	Codon		Anti-codon
			Start	End				Start	Stop	
nad5	NADH dehydrogenase subunit 5	PCG	205	1899	1695	–	-12	att	tag	–
trnH	Transfer RNA for Histidine	tRNA	1888	1948	61	–	10	–	–	gtg
nad4	NADH dehydrogenase subunit 4	PCG	1959	2966	1008	–	292	att	t	–
nad4L	NADH dehydrogenase subunit 4L	PCG	3259	3531	273	–	26	atg	taa	–
trnC	Transfer RNA for Cysteine	tRNA	3558	3617	60	+	31	–	–	gca
nad6	NADH dehydrogenase subunit 6	PCG	3649	4122	474	+	51	att	taa	–
rrnL	16S ribosomal RNA	rRNA	4174	5325	1152	+	22	–	–	–
COX1	cytochrome c oxidase subunit I	PCG	5348	6889	1542	+	65	ata	taa	–
nad3	NADH dehydrogenase subunit 3	PCG	6955	7305	351	+	-1	att	taa	–
COX2	cytochrome c oxidase subunit II	PCG	7305	7964	660	+	11	ata	taa	–
trnG	Transfer RNA for Glycine	tRNA	7976	8037	62	+	3	–	–	tcc
trnK	Transfer RNA for Lysine	tRNA	8041	8102	62	+	10	–	–	ttt
COX3	cytochrome c oxidase subunit III	PCG	8113	8895	783	+	0	ata	taa	–
trnN	Transfer RNA for Asparagine	tRNA	8896	8959	64	+	-3	–	–	gtt
trnT	Transfer RNA for Threonine	tRNA	8957	9019	63	+	-1	–	–	tgt
trnL1	Transfer RNA for Leucine	tRNA	9019	9082	64	+	2	–	–	tag

(Continued)

TABLE 1 Continued

Gene Name	Full Name and Function	Gene Type	Position		Length (bp)	Strand	Intergenic Space	Codon		Anti-codon
			Start	End				Start	Stop	
trnE	Transfer RNA for Glutamic acid	tRNA	9085	9147	63	+	8	–	–	ttc
trnS1	Transfer RNA for Serine	tRNA	9156	9212	57	+	6	–	–	tct
trnQ	Transfer RNA for Glutamine	tRNA	9219	9286	68	+	37	–	–	ttg
trnS2	Transfer RNA for Serine	tRNA	9324	9387	64	+	0	–	–	tga
trnD	Transfer RNA for Aspertic acid	tRNA	9388	9451	64	+	451	–	–	gtc
trnR	Transfer RNA for Arginine	tRNA	9903	9968	66	–	18	–	–	tcg
ATP6	ATP synthase F0 subunit 6	PCG	9987	10607	621	–	50	ata	taa	–
ATP8	ATP synthase F0 subunit 8	PCG	10658	10860	203	–	-54	att	t	–
rrnS	12S ribosomal RNA	rRNA	10807	11413	607	–	116	–	–	–
trnF	Transfer RNA for Phenylalanine	tRNA	11530	11594	65	–	-1	–	–	gaa
trnA	Transfer RNA for Alanine	tRNA	11594	11656	63	–	2	–	–	tgc
trnM	Transfer RNA for Methionine	tRNA	11659	11721	63	–	-4	–	–	cat
nad1	NADH dehydrogenase subunit 2	PCG	11718	12641	924	–	6	ata	taa	–
nad2	NADH dehydrogenase subunit 1	PCG	12648	13628	981	–	73	att	taa	–
trnY	Transfer RNA for Tyrosine	tRNA	13702	13765	64	+	-3	–	–	gta
CYTB	cytochrome b	PCG	13763	14908	1146	–	-29	ata	tag	–
trnI	Transfer RNA for Isoleucine	tRNA	14880	14943	64	–	2	–	–	gat
trnP	Transfer RNA for Proline	tRNA	14946	15008	63	+	268	–	–	tgg

in *T. parvispinus*, the coding sequences ranged from 160 bp (atp8) to 1,695 bp (nad5), with most genes situated on the H-strand, except nad4, nad4L, and nad5, which were on the L-strand.

Three start codons (ATT, ATA, and ATG) were identified in the *T. tabaci* mitogenome. ATT was adopted by genes like nad2, nad3, nad4, nad5, nad6, and atp8, whereas genes like cox1, cox2, cox3, atp6, nad2, and CytB used ATA. However, ATG was used as the start codon by the nad4L gene. In contrast, *T. parvispinus* utilized four start codons, with ATT being the most prevalent across 8 genes (cox1, atp6, atp8, nad1, nad2, nad3, nad5, and nad6), while 3 genes (nad4, cox2, and cytB) used ATA as their start codon. The ATG and TTG start codons are used by nad4L and cox3, respectively. The ATN start codon is the most common starting codon in most thrips and other insect species (26, 47–49). For stop codons, *T. tabaci* primarily used TAA, but nad5 and cytB used TAG, and nad4 and atp8 used an incomplete stop codon "T". Similarly, *T. parvispinus* mostly used TAA, with nad4 and atp8 also adopting the incomplete "T". Incomplete termination codons are common in other thrips species (26, 47) and insect mitochondrial genomes and are presumed to be restored through post-transcriptional polyadenylation (48, 49).

The RSCU and amino acid usage in the PCGs of *T. tabaci* and *T. parvispinus* are summarized in Figure 2 and Table 5. In *T. tabaci* mitogenome, leucine, phenylalanine, isoleucine, serine, lysine, tyrosine, and valine were the most frequent amino acids, whereas tryptophan, methionine, and cyanine were the rarest. In general, UUU, followed by UUA, AUU, AUA, and AAA, was the most frequently used codon. However, in the *T. parvispinus* mitogenome, isoleucine, phenylalanine, leucine, asparagine, serine, lysine, and tyrosine were the most frequently used amino acids, whereas tryptophan, cyanine, and methionine were the rarest, with UUU, followed by AUU, UUA, AAU, and AUA being the most frequently used codons (Table 5).

Transfer RNAs and ribosomal RNAs

The circular mitochondrial genome of *T. tabaci* contains 19 tRNA genes ranging in size from 57 to 68 bp. In the mitogenome, 13 of the tRNA genes are located on the H-strand (+), while the remaining six are located on the L-strand (–) (Figure 1A and Table 1). The 19

TABLE 2 Nucleotide composition of *Thrips tabaci* mitogenome.

Gene	T	C	A	G	Total	GC%	AT%	GC Skew	AT Skew
nad5	53.2	10.9	25.2	10.7	1695	21.60	78.40	-0.009	-0.357
nad4	51.8	10.8	24.4	13	1008	23.80	76.20	0.092	-0.360
nad4l	56.4	8.8	22.7	12.1	273	20.90	79.10	0.158	-0.426
nad6	44.7	9.9	37.8	7.6	474	17.50	82.50	-0.131	-0.084
cox1	37.5	15.9	32.3	14.3	1542	30.20	69.80	-0.053	-0.074
nad3	38.7	12.9	38.7	9.7	351	22.60	77.40	-0.142	0.000
cox2	35.6	16.2	36.2	12	660	28.20	71.80	-0.149	0.008
cox3	39.4	16.4	32.4	11.8	783	28.20	71.80	-0.163	-0.097
atp6	37.7	14.8	36.2	11.3	621	26.10	73.90	-0.134	-0.020
atp8	41.3	14.3	32.5	11.9	126	26.20	73.80	-0.092	-0.119
nad1	37.3	15.4	35.5	11.8	924	27.20	72.80	-0.132	-0.025
nad2	39.1	12	41.8	7.1	981	19.10	80.90	-0.257	0.033
cob	40.6	14.7	32.7	12	1146	26.70	73.30	-0.101	-0.108
rrnS	45.8	12.2	31	11	607	23.20	76.80	-0.052	-0.193
rrnL	32.5	9.8	46.8	10.9	1152	20.70	79.30	0.053	0.180

TABLE 3 Summary of *Thrips parvispinus* mitochondrial genome.

Gene Name	Full Name and Function	Gene Type	Position		Length (bp)	Strand	Intergenic Space	Codon		Anti-codon
			Start	End				Start	Stop	
nad5	NADH dehydrogenase subunit 5	PCG	146	1840	1695	–	-5	att	taa	–
nad4	NADH dehydrogenase subunit 4	PCG	1836	3157	1322	–	47	ata	t	–
nad4L	NADH dehydrogenase subunit 4L	PCG	3205	3486	282	–	25	atg	taa	–
trnC	Transfer RNA for Cysteine	tRNA	3512	3574	63	+	18	–	–	gca
nad6	NADH dehydrogenase subunit 6	PCG	3593	4090	498	+	100	att	taa	–
rrnL	16S ribosomal RNA	rRNA	4191	5338	1148	+	20	–	–	–
cox1	cytochrome c oxidase subunit I	PCG	5359	6930	1572	+	71	att	taa	–
nad3	NADH dehydrogenase subunit 3	PCG	7002	7343	342	+	-35	att	taa	–
cox2	cytochrome c oxidase subunit II	PCG	7309	8001	693	+	23	ata	taa	–
trnG	Transfer RNA for Glycine	tRNA	8025	8087	63	+	3	–	–	tcc
trnK	Transfer RNA for Lysine	tRNA	8091	8154	64	+	2	–	–	ttt
cox3	cytochrome c oxidase subunit III	PCG	8157	8945	789	+	4	ttg	taa	–

(Continued)

TABLE 3 Continued

Gene Name	Full Name and Function	Gene Type	Position		Length (bp)	Strand	Intergenic Space	Codon		Anti-codon
			Start	End				Start	Stop	
trnN	Transfer RNA for Asparagine	tRNA	8950	9016	67	+	-4	–	–	gtt
trnS1	Transfer RNA for Serine	tRNA	9013	9073	61	+	0	–	–	tct
trnL1	Transfer RNA for Leucine	tRNA	9074	9138	65	+	42	–	–	tag
trnE	Transfer RNA for Glutamic acid	tRNA	9181	9246	66	+	347	–	–	ttc
trnP	Transfer RNA for Proline	tRNA	9594	9659	66	–	34	–	–	tgg
trnY	Transfer RNA for Tyrosine	tRNA	9694	9757	64	–	57	–	–	gta
nad2	NADH dehydrogenase subunit 2	PCG	9815	10825	1011	+	17	att	taa	–
nad1	NADH dehydrogenase subunit 1	PCG	10843	11772	930	+	-4	att	taa	–
trnM	Transfer RNA for Methionine	tRNA	11769	11833	65	+	1	–	–	cat
trnA	Transfer RNA for	tRNA	11835	11896	62	+	-1	–	–	tgc
trnF	Transfer RNA for Phenylalanine	tRNA	11896	11960	65	+	-2	–	–	gaa
rrnS	12S ribosomal RNA	rRNA	11959	12711	753	+	8	–	–	–
atp8	ATP synthase F0 subunit 8	PCG	12720	12879	160	+	41	att	t	–
atp6	ATP synthase F0 subunit 6	PCG	12921	13535	615	+	5	att	taa	–
trnQ	Transfer RNA for Glutamine	tRNA	13541	13609	69	+	59	–	–	ttg
trnS2	Transfer RNA for Serine	tRNA	13669	13733	65	+	3	–	–	tga
trnD	Transfer RNA for Aspertic acid	tRNA	13737	13801	65	+	1	–	–	gtc
trnR	Transfer RNA for Arginine	tRNA	13803	13870	68	+	0	–	–	tcg
trnT	Transfer RNA for Threonine	tRNA	13871	13935	65	+	10	–	–	tgt
trnI	Transfer RNA for Isoleucine	tRNA	13946	14012	67	+	-32	–	–	gat
cytB	cytochrome b	PCG	13981	15126	1146	+	101	ata	taa	–

tRNA-coding genes of *T. tabaci* mitochondrial genome collectively comprised 1,200 bp, representing 7.85% of the entire mitogenome. In *T. parvispinus* mitogenome, 18 tRNA-coding genes ranging in size from 61 to 69 bp were detected. All of the tRNA-coding genes of *T. parvispinus* were located on the H-strand (+) with the exception of two genes encoding proline (Pro/P) and tyrosine (Tyr/Y) located on the L-strand (-) of mitogenome (Figure 1B and Table 3). These 18 tRNA-coding genes of *T. parvispinus* comprise a 1,170-bp region that covers 7.65% of the mitogenome. The characteristic clover leaf secondary structures of tRNA genes were identified in the

mitogenomes of both thrips species, with the exception of trnS1, which lacks a dihydrouridine arm (Figures 3, 4). Three genes encoding tRNAs for leucine (Leu/L2), tryptophan (Trp/W), and valine (Val/V) were lacking in the mitogenome of *T. tabaci*; however, in addition to these three genes, histidine (His/H) was missing in the *T. parvispinus* mitogenome.

The rRNA genes rrnL and rrnS collectively constitute 11.51% of the total mitogenome. The rrnS gene (607 bp long with 76.80% A+T content) was situated between the atp8 and tRNA-Phe genes. The rrnL gene (1,152 bp long with 79.30% A+T content) was located between the

TABLE 4 Nucleotide composition of *Thrips parvispinus* mitochondrial genome.

| Gene	Length	T	C	A	G	GC%	AT%	GC Skew	AT Skew
nad5	1695	54.65	8.40	26.35	10.60	19.00	81.00	0.116	-0.349
nad4	1322	55.45	7.60	25.25	11.70	19.30	80.70	0.212	-0.374
nad4l	282	59.20	6.75	24.80	9.25	16.00	84.00	0.156	-0.410
nad6	498	40.40	9.60	42.00	8.00	17.60	82.40	-0.091	0.019
cox1	1572	36.40	14.20	36.40	13.00	27.20	72.80	-0.044	0.000
nad3	342	38.60	11.70	40.90	8.80	20.50	79.50	-0.141	0.029
cox2	693	34.50	14.30	39.20	12.00	26.30	73.70	-0.087	0.064
cox3	789	36.75	15.50	36.45	11.30	26.80	73.20	-0.157	-0.004
nad2	1011	40.50	11.60	42.50	5.40	17.00	83.00	-0.365	0.024
nad1	930	40.75	12.85	35.40	11.00	23.85	76.15	-0.078	-0.070
atp8	160	38.10	11.30	42.50	8.10	19.40	80.60	-0.165	0.055
atp6	615	39.20	13.30	37.10	10.40	23.70	76.30	-0.122	-0.028
cob	1146	40.00	13.75	35.10	11.15	24.90	75.10	-0.104	-0.065
rrnL	1148	32.35	9.10	47.25	11.30	20.40	79.60	0.108	0.187
rrnS	753	33.30	10.40	46.10	10.20	20.60	79.40	-0.010	0.161
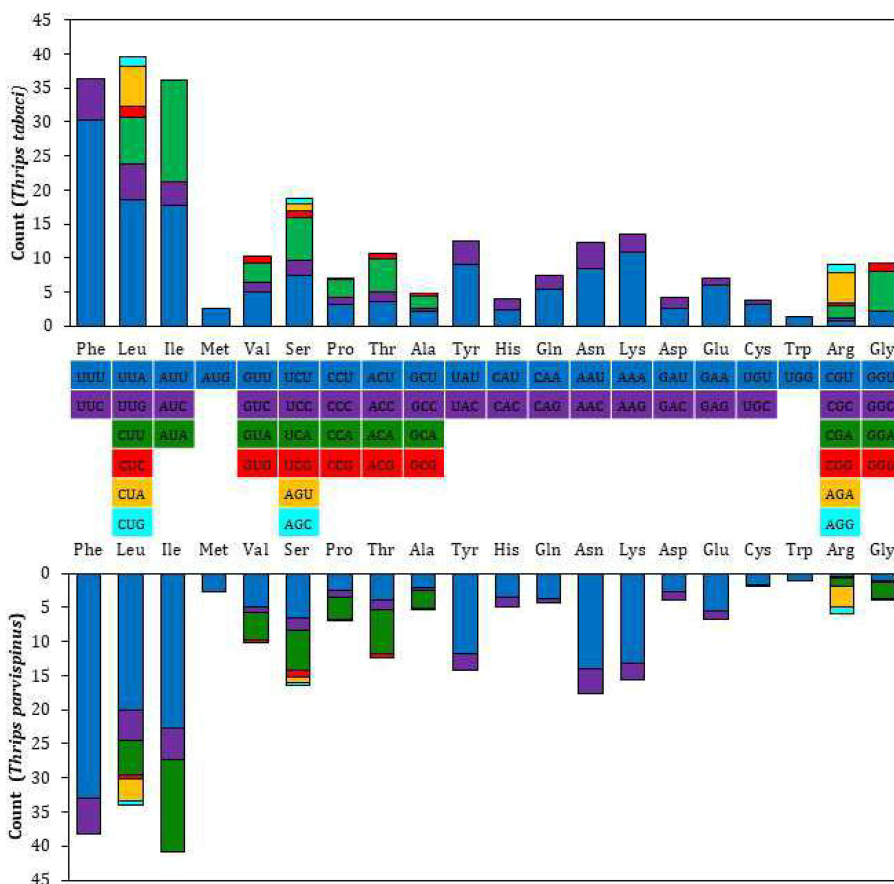
FIGURE 2
Relative synonymous codon use (RSCU) of 13 PCG's of the *T. tabaci* and *T. parvispinus*.

TABLE 5 Amino acid usage in the 13 PCG's of the *T. tabaci* and *T. parvispinus*.

Codon	*T. tabaci*	*T. parvispinus*	Codon	*T. tabaci*	*T. parvispinus*	Codon	*T. tabaci*	*T. parvispinus*	Codon	*T. tabaci*	*T. parvispinus*
UUU(F)	30.3	33	UCU(S)	7.5	6.6	UAU(Y)	9	11.8	UGU(C)	3.1	2.6
UUC(F)	6.3	5.2	UCC(S)	2.2	1.8	UAC(Y)	3.5	2.5	UGC(C)	0.6	0.6
UUA(L)	18.5	20.1	UCA(S)	6.2	5.8	UAA(*)	7	5.7	UGA(*)	4.6	4.1
UUG(L)	5.3	4.4	UCG(S)	1.1	1.1	UAG(*)	2.3	1.9	UGG(W)	1.3	1.2
CUU(L)	6.9	5.1	CCU(P)	3.2	2.6	CAU(H)	2.3	3.5	CGU(R)	0.8	1
CUC(L)	1.7	0.5	CCC(P)	0.9	1	CAC(H)	1.7	1.4	CGC(R)	0.3	0.2
CUA(L)	5.7	3.2	CCA(P)	2.8	3.2	CAA(Q)	5.5	3.8	CGA(R)	1.8	2.2
CUG(L)	1.5	0.7	CCG(P)	0.2	0.2	CAG(Q)	2	0.6	CGG(R)	0.5	0.2
AUU(I)	17.8	22.6	ACU(T)	3.5	3.9	AAU(N)	8.5	14.1	AGU(S)	1	2.2
AUC(I)	3.4	4.8	ACC(T)	1.5	1.5	AAC(N)	3.7	3.6	AGC(S)	0.7	1.4
AUA(I)	14.9	13.5	ACA(T)	4.8	6.3	AAA(K)	10.8	13.2	AGA(R)	4.4	5.8
AUG(M)	2.5	2.8	ACG(T)	0.9	0.7	AAG(K)	2.6	2.5	AGG(R)	1.3	1.9
GUU(V)	5.1	5	GCU(A)	2.1	2.2	GAU(D)	2.6	2.8	GGU(G)	2.1	2.5
GUC(V)	1.4	0.8	GCC(A)	0.4	0.3	GAC(D)	1.5	1.1	GGC(G)	0.1	0.2
GUA(V)	2.8	3.9	GCA(A)	1.8	2.6	GAA(E)	6	5.5	GGA(G)	5.9	5.5
GUG(V)	1	0.4	GCG(A)	0.5	0.2	GAG(E)	1	1.2	GGG(G)	1.2	0.5

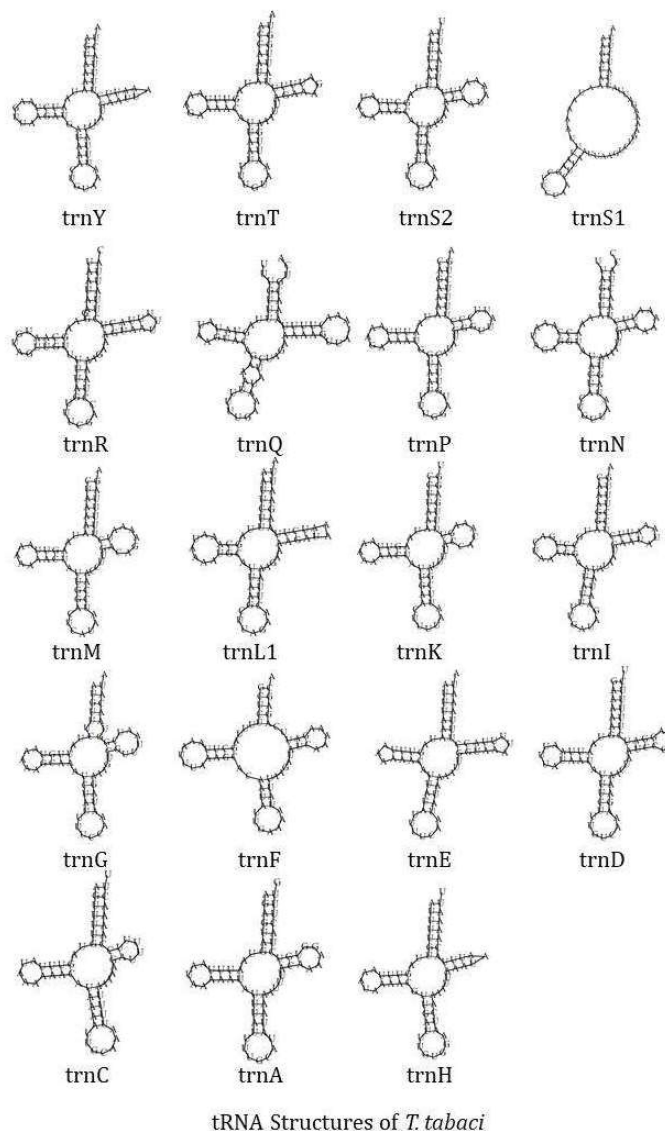The symbol * represent the "Stop codons".

FIGURE 3
tRNA structures of the *T. tabaci*.

nad6 and cox1 genes of the *T. tabaci* mitogenome. However, in the *T. parvispinus* mitogenome, both the rRNA-coding genes rrnL (1148 bp) and rrnS (753 bp) were found on the H-strand (+) and covered 12.43% (1901 bp) of the whole mitochondrial genome.

Phylogenetic analysis

Species within the same family, Thripidae, *T. palmi*, *T. imagines*, *T. hawaiiensis*, F. *intonsa*, *F. occidentalis*, *Dendrothrips minowai*, *Pseudodendrothrips mori*, *Neohydatothrips samayunkur*, *Scirtothrips dorsalis*, *Anaphothrips obscurus*, were grouped together with *T. tabaci* and *T. parvispinus*, The species from other thrips family such as Stenurothripidae, Aeolothripidae and Phlaeothripidae were grouped in another cluster. The *T. parvispinus* was closely related to *T. hawaiiensis* with 98 bootstrap

value. However, *T. tabaci* was slightly outgrouped from *T. hawaiiensis*. The *F. intonsa*, and *F. occidentalis* were cluster separately from species for genus *Thrips* (Figure 5). The other cluster included *Holarthothrips indicus* of the Stenurothripidae family grouped with 51 Bootstrap value with *Franklinothrips vespiformis* of the Aeolothripidae family, *Gynaikothrips uzeli*, and *Haplothrips aculeatus* of the Phlaeothripidae family. The damsel bug, *Alloeorhynchus bakeri*, was used in the dataset as an outgroup species.

Control, overlapping, and intergenic spacer regions

The *T. tabaci* and *T. parvispinus* mitochondrial genome contains one control in both species, each of which is 451 bp and 347 bp,
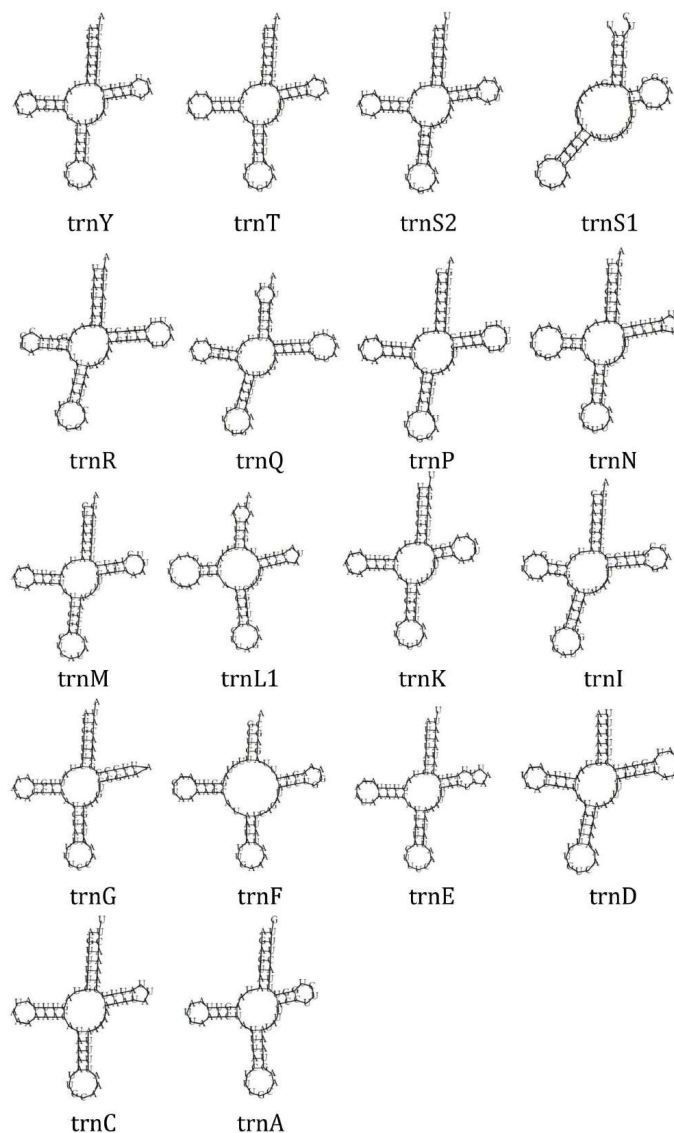
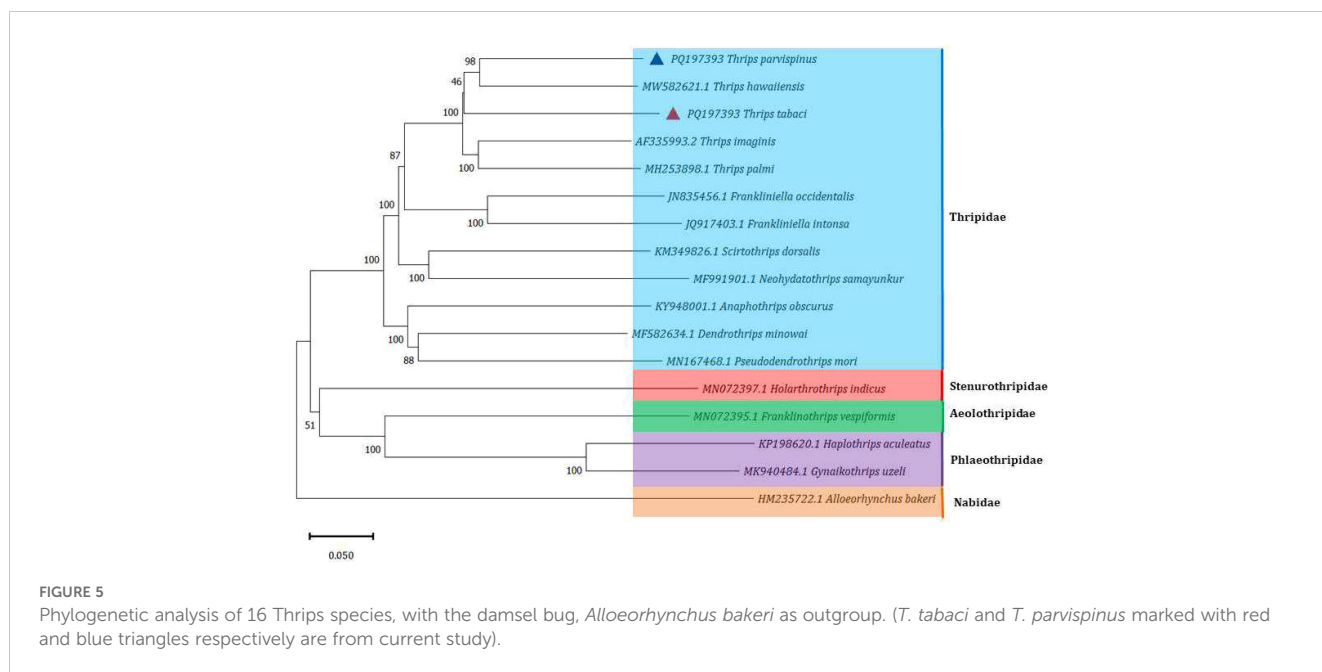tRNA structures of the *T. parvispinus*.

respectively. In *T. tabaci*, it is located between the trnD and trnR genes; however, in *T. parvispinus*, it is situated between the trnE and trnP genes. The percentages of AT in the control *T. tabaci* and *T. parvispinus* regions were 62.7% and 76.7%, respectively. T-stretch, GAnT motif, ATnC motif, tandem repeats, and stem loops were found in the control region of the *T. tabaci* mitogenome. However, the T-stretch followed by the TATA box, GAnT motif, tandem repeats, and stem loop were found in the *T. parvispinus* mitogenome.

The *T. tabaci* mitochondrial genomes contain 22 intergenic spacer regions of length 1109 bp that vary in length from 2 to 292 bp. There were 13 major intergenic spacers >10 bp in length observed in the circular genome. The longest intergenic spacer regions (292 bp) were located between nad4 and nad4L genes. However 9 overlapping sequences were found in *T. tabaci* mitochondrial genome, ranging in size from 1 to 54 bp. Similar

to *T. tabaci*, *T. parvispinus* mitochondrial genome contained 22 intergenic spacer regions of total 692 bp, varying in length from 1 to 101 bp. There were 14 major intergenic spacers >10 bp in length observed in the *T. parvispinus* mitogenome. The cytB genes were followed by the longest intergenic spacer region of 101 bp. The 7 overlapping sequences were present in *T. tabaci* mitochondrial genome, ranging from 1 to 35 bp in size. Amid the nad4 and nad4L genes of the *T. tabaci* mitogenome, there were 292 bp noncoding nucleotides; however, *T. parvispinus* revealed 47 bp.

Discussion

The circular mitogenome of *T. tabaci* and *T. parvispinus* from the present study revealed 15,277 bp and 15,285 bp. respectively. A

FIGURE 5
Phylogenetic analysis of 16 Thrips species, with the damsel bug, *Alloeorhynchus bakeri* as outgroup. (*T. tabaci* and *T. parvispinus* marked with red and blue triangles respectively are from current study).

similar trend was observed in most of the thrips species reported earlier. For instance, *T. imagines* (15,407 bp) [21], *T. palmi* (15,333 bp) [26], *Frankliniella intonsa* (15,215 bp) [23], *Frankliniella occidentalis* (14,889 bp) [22], *Scriptothrips dorsalis* (15,343 bp) [24], and *Anaphothrips obscurus* (14,890 bp) [25].

The gene order among these thrips species showed variations, especially within the subfamily Thripinae. In *T. tabaci* and *T. parvispinus* the several tRNA genes from both *T. tabaci* and *T. parvispinus* have been translocated, showing the variation and arrangement of the gene. Similar variation in the order of the mitogenome genes of the all thrips species was observed. This variation can provide insights into evolutionary processes and mechanisms of genetic diversity. It also highlights the complexity of mitochondrial inheritance and their adaptation to various environments at the genetic level [47]. These variations might affect their physiology, behavior, and ecological interactions, making them an interesting subject for evolutionary studies [48]. Additionally, understanding these evolutionary patterns can help in pest control strategies, as some thrips species are significant agricultural pests.

The RSCU data analysis revealed that Lysine followed by phenylalanine, leucine, isoleucine, tyrosine, and serine, are the most frequently used amino acids, which is common in most of the thrips species [23, 25, 26]. The characteristic clover leaf secondary structures of tRNA genes were identified in the mitogenomes of both thrips species, with the exception of trnS1, which lacks a dihydrouridine (DHU) arm, which is common in most insect species. The DHU arm in the trnS1 secondary structure was missing in marigold thrips (*Neohydatothrips samayunkur*) [49], green semilooper (*Chrysodeixis acutaI*) [50], and Indian dammer bee, *Tetragonula iridipennis* [51].

In both the thrips species, some tRNA-coding genes, namely Leucine (Leu/L2), Tryptophan (Trp/W), and Valine (Val/V) were missing in *T. tabaci*, and in addition to these three, Histidin (His/H)

is missing in *T. parvispinus* mitogenome. The presence of duplicate copies of tRNA in some thrips species and missing tRNA genes in Gynaikothrips has been reported by Tyagi et al. [52]. However in many insect species mitochondrialt tRNA genes reported as lost or missing, on manual annotation found to have unusual secondary structures and contain many nucleotide mismatches [53]. In some of the insect species, the truncated tRNA was observed, which formed during their evolution [54]. The truncation of tRNA genes poses problems in locating and annotating them due to a high level of nucleotide mismatches [55]. In the absence of a well-paired acceptor stem, the 3' end is not clearly defined. The region downstream from the anticodon stem is extremely variable in sequence and length [54].

The 7 overlapping sequences were present in the *T. tabaci* mitochondrial genome, ranging from 1 to 35 bp in size. Amid the nad4 and nad4L genes of the *T. tabaci* mitogenome, there were 292 bp noncoding nucleotides; however, *T. parvispinus* revealed 47 bp, as in *Aeolothrips xinjiangensis* (148 bp) [48]. Most of the thrips mitogenomes exhibited overlaps of 1-21 bp (mostly around 7 bp) between the nad4 and nad4L. In insect mitogenomes, the nd4 to nd4L regions are transcribed into polycistronic mRNA with either overlaps or no intergenic spacers between them [56–58]. However, such a long intergenic spacer might split this polycistronic mRNA into two monocistronic mRNAs [48].

The phylogenetic analysis revealed that the genera Thrips and Frankliniella are closely related, as they cluster together. This close relationship is attributed to their shared homology of paired ctenidia on abdominal segments V-VIII [59]. *T. tabaci*, *T. palmi*, *F. accidentalis*, and *F. intonsa* are known vectors of topoviruses [47]. These species cluster together, suggesting they share similar genomic characteristics.

As like the genomic characteristics, the biological similarities are also present in *T. tabaci* and *T. parvispinus*. Field emission scanning electron microscopy (FESEM) analysis of both thrips

species identified similar types of sensilla, including sensilla basiconica (SBI, SBII, SBIII), sensilla chaetica (SChI, SChII), sensilla trichodea (ST), sensilla campaniformia (SCa), and sensilla cavity (SCav); however, variations in the length of these sensilla were observed between the two species. Also, some morphological characters have the variation in both the thrips species, such as antennae (seven-segmented with forked sensorium on third, and fourth segments), ctenidia (paired ctenidia were present in 5th–8th abdominal segments laterally), and pronotum (two pairs of posteroangular setae) (60).

Mitochondrial genome data have been widely used for phylogenetic, evolutionary studies, and population genetics in insects (61). Among eukaryotes, Thysanoptera, along with other minor paraenopteran insect orders, is regarded as a model for rapid mitochondrial genome evolution (24). In the Thysanoptera order, thrips display exceptional interspecific variation in mitogenomic structure, making them an ideal model for studying mitochondrial evolution (48). In the current study, the several tRNA genes from both *T. tabaci* and *T. parvispinus* have been translocated; however, the protein-coding genes and most of the tRNA genes have a similar arrangement, representing that these two species have a low rate of the rearrangement among them, which may be originating from the most recent common ancestors of these thrips species. Similar results were reported by Yan et al. (23) in flower thrips, *Frankliniella intonsa*, and other thrips species. Mitochondrial DNA plays a crucial regulatory role in insect adaptation to environmental changes, including insecticide resistance (62). Many insecticides target mitochondrial functions, such as oxidative phosphorylation. Mutations in mtDNA can lead to changes in these functions, resulting in resistance to the insecticides (63). Mutations in the mitochondrial-encoded Cytb gene have been implicated in resistance to the novel acaricide Bifenazate in *Tetranychus urticae* and *Panonychus citri* (64). Furthermore, a newly identified mutation in the Cytb gene of *T. urticae* has been associated with resistance to the miticide Acequinocyl (65). However, in the current study, no such mutation was detected in the mitochondrial genes. Also, no report was found in any thrips species to date detecting insecticidal resistance in the thrips species due to mutation in the mitochondrial gene.

The phylogenetic analysis includes species from different thrips families, such as Thripidae, Aeolothripidae, Phlaeothripidae, and Stenurothripidae, showing the phylogenetic diversity among these groups. The diversity within these groups may contribute to the complexity and lower bootstrap values in some parts of the tree. The thrips species clustered together such as *T. hawaiiensis* and *T. parvispinus*, with bootstrap values 98, have closely related species *T. tabaci* with lowest bootstrap value 46, suggesting lower confidence in their relationships. The thrips species in phylogenetic study other than the Thripidae family, *Holarthrothrips indicus* of Stenurothripidae family, and *Franklinothrips vespiformis* of Aeolothripidae, *Gynaikothrips uzeli* and *Haplothrips aculeatus* of the family Phlaeothripidae shown the lower boot strap values, 51. The lower bootstrap values of these species could be because of the low rate of genetic flow between these species (66).

Conclusion

Complete mitochondrial genome sequences of *T. tabaci* and *T. parvispinus* provide critical insights into their genetic makeup and evolutionary relationships. The mitochondrial genomes of *T. tabaci* and *T. parvispinus* were found to be 15,277 bp and 15,285 bp in size, respectively, with similar gene organization, including 13 protein-coding genes, 2 rRNA genes, and 19 tRNA genes in *T. tabaci*, and 18 tRNA genes in *T. parvispinus*. The high AT content observed in both species reflects their typical insect mitochondrial genome characteristics, which are essential for understanding their genetic diversity and evolutionary adaptations. Phylogenetic analysis revealed the evolutionary positions of *T. tabaci* and *T. parvispinus* within the Thysanoptera order, highlighting the genetic makeup and taxonomy of these species with other thrips species. The findings emphasize the significance of understanding the genetic basis of onion thrips, which can aid in developing targeted pest management strategies to mitigate their impact on onion production. The detailed mitochondrial genome sequences and phylogenetic analyses presented in this study provide a valuable resource for further research on thrips biology, genetics, and pest management.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

Funding

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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References

1. Sharma K, Mahato N, Nile SH, Lee ET, Lee YR. Economical and environmentally-friendly approaches for usage of onion (Allium cepa L.) waste. *Food Funct*. (2016) 7:3354–69. doi: 10.1039/C6FO00251J

2. Gulati A, Wardhan H, Sharma P. Tomato, onion and potato (TOP) value chains. In: Gulati A, Ganguly K, Wardhan H, editors. *Agricultural value chains in India: ensuring competitiveness, inclusiveness, sustainability, scalability, and improved finance.* Singapore: Springer Nature Singapore (2022). p. 33–97. Available at: https://library.oapen.org/handle/20.500.12657/52430 (Accessed October 10, 2024).

3. Leach A, Reiners S, Nault B. Challenges in integrated pest management: A case study of onion thrips and bacterial bulb rot in onion. *Crop Prot*. (2020) 133:105123. doi: 10.1016/j.cropro.2020.105123

4. Nawrocka B, Vidal S. Economic importance and the control method of Thrips tabaci Lind. on onion. *IOBC WPRS Bull*. (2003) 26:321–4.

5. Diaz-Montano J, Fuchs M, Nault BA, Fail J, Shelton AM. Onion thrips (Thysanoptera: Thripidae): a global pest of increasing concern in onion. *J economic entomology*. (2011) 104:1–13. doi: 10.1603/EC10269

6. Gill HK, Garg H, Gill AK, Gillett-Kaufman JL, Nault BA. Onion thrips (Thysanoptera: Thripidae) biology, ecology, and management in onion production systems. *J Integrated Pest Manage*. (2015) 6:6. doi: 10.1093/jipm/pmv006

7. Soumia PS, Karuppaiah V, Singh M. Managing Thrips tabaci: a pest of national significance. *Indian Horticulture*. (2017) 62:55–6. https://epubs.icar.org.in/index.php/IndHort/article/view/87714 (Accessed October 10, 2024).

8. Khandagale K, Roylawar P, Randive P, Karuppaiah V, Soumia PS, Shirsat D, et al. Isolation and expression profiling of insecticidal lectins from wild alliums against onion thrips (Thrips tabaci lindeman). *Proc Natl Acad Sciences India Section B: Biol Sci*. (2022) 92:451–9. doi: 10.1007/s40011-022-01360-4

9. Iglesias L, Havey MJ, Nault BA. Management of onion thrips (Thrips tabaci) in organic onion production using multiple IPM tactics. *Insects*. (2021) 12:207. doi: 10.3390/insects12030207

10. Gawande SJ, Anandhan S, Ingle A, Roylawar P, Khandagale K, Gawai T, et al. Microbiome profiling of the onion thrips, Thrips tabaci Lindeman (Thysanoptera: Thripidae). *PloS One*. (2019) 14:e0223281. doi: 10.1371/journal.pone.0223281

11. Leach A, Hay F, Harding R, Damann KC, Nault B. Relationship between onion thrips (Thrips tabaci) and Stemphylium vesicarium in the development of Stemphylium leaf blight in onion. *Ann Appl Biol*. (2020) 176:55–64. doi: 10.1111/aab.12558

12. Grode A, Chen S, Walker ED, Szendrei Z. Onion thrips (Thysanoptera: Thripidae) feeding promotes infection by Pantoea ananatis in onion. *J economic entomology*. (2017) 110:2301–7. doi: 10.1093/jee/tox273

13. Grode AS, Brisco-McCann E, Wiriyajitsonboom P, Hausbeck MK, Szendrei Z. Managing onion thrips can limit bacterial stalk and leaf necrosis in Michigan onion fields. *Plant Dis*. (2019) 103:938–43. doi: 10.1094/PDIS-07-18-1271-RE

14. Khan AA, Kanwal H, Batool S, Hassan Z, Shah JM, Aatif HM, et al. Impact of Predators and Some Commercial Insecticides on Thrips tabaci Lindeman and Amrasca biguttula Ishida Populations in Bt Cotton under Arid Climate. *Pakistan J Zoology*. (2022) 54:137–44. doi: 10.17582/journal.pjz/20200512190543

15. Khan F, Roy MC, Kim Y. Thelytokous reproduction of onion thrips, Thrips tabaci Lindeman 1889, infesting welsh onion and genetic variation among their subpopulations. *Insects*. (2022) 13:78. doi: 10.3390/insects13010078

16. Karuppaiah V, Maruthadurai R, Das B, Soumia PS, Gadge AS, Thangasamy A, et al. Predicting the potential geographical distribution of onion thrips, Thrips tabaci in India based on climate change projections using MaxEnt. *Sci Rep*. (2023) 13:7934. doi: 10.1038/s41598-023-35012-y

17. Morse JG, Hoddle MS. Invasion biology of thrips. *Annu Rev entomology*. (2006) 51:67–89. doi: 10.1146/annurev.ento.51.110104.151044

18. Martin NA, Workman PJ, Butler RC. Insecticide resistance in onion thrips (Thrips tabaci)(Thysanoptera: Thripidae). *New Z J Crop Hortic Sci*. (2003) 31:99–106. doi: 10.1080/01140671.2003.9514242

19. Saini S, Raj K, Saini AK. Effect of Thrips parvispinus injury in enhancing onion purple blotch severity. *Plant Dis Res*. (2022) 37:246–6.

20. Saini S, Raj K, Saini AK, Kumar R, Saini A, Khan A, et al. Unravelling the synergistic interaction of Thrips tabaci and newly recorded, Thrips parvispinus with Alternaria porri (Ellis.) Cif., inciting onion purple blotch. *Front Microbiol*. (2024) 15:1321921. doi: 10.3389/fmicb.2024.1321921

21. Shao R, Barker SC. The highly rearranged mitochondrial genome of the plague thrips, Thrips imaginis (Insecta: Thysanoptera): convergence of two novel gene boundaries and an extraordinary arrangement of rRNA genes. *Mol Biol Evol*. (2003) 20:362–70. doi: 10.1093/molbev/msg045

22. Yan D, Tang Y, Xue X, Wang M, Liu F, Fan J. The complete mitochondrial genome sequence of the western flower thrips Frankliniella occidentalis (Thysanoptera: Thripidae) contains triplicate putative control regions. *Gene*. (2012) 506:117–24. doi: 10.1016/j.gene.2012.06.022

23. Yan D, Tang Y, Hu M, Liu F, Zhang D, Fan J. The mitochondrial genome of Frankliniella intonsa: insights into the evolution of mitochondrial genomes at lower taxonomic levels in Thysanoptera. *Genomics*. (2014) 104:306–12. doi: 10.1016/j.ygeno.2014.08.003

24. Dickey AM, Kumar V, Morgan JK, Jara-Cavieres A, Shatters RG, McKenzie CL, et al. A novel mitochondrial genome architecture in thrips (Insecta: Thysanoptera): extreme size asymmetry among chromosomes and possible recent control region duplication. *BMC Genomics*. (2015) 16:1–7. doi: 10.1186/s12864-015-1672-4

25. Liu H, Li H, Song F, Gu W, Feng J, Cai W, et al. Novel insights into mitochondrial gene rearrangement in thrips (Insecta: Thysanoptera) from the grass thrips, Anaphothrips obscurus. *Sci Rep*. (2017) 7:4284. doi: 10.1038/s41598-017-04617-5

26. Chakraborty R, Tyagi K, Kundu S, Rahaman I, Singha D, Chandra K, et al. The complete mitochondrial genome of Melon thrips, Thrips palmi (Thripinae): Comparative analysis. *PloS One*. (2018) 13:e0199404. doi: 10.1371/journal.pone.0199404

27. Chen SC, Jiang HY, Peng P, Hu X, Lin Q, Wang XQ. The complete mitochondrial genome of the stick tea thrips Dendrothrips minowai (Thysanoptera: Thripidae). *Mitochondrial DNA Part B*. (2018) 3:58–9. doi: 10.1080/23802359.2017.1419099

28. Wang Y, Wu C, Xie Y, Liu H, Zhang H. Complete mitochondrial genome sequence for the Thrips hawaiiensis (Thysanoptera: Thripidae). *Mitochondrial DNA Part B*. (2021) 6:2092–4. doi: 10.1080/23802359.2021.1942268

29. Pakrashi A, Patidar A, Singha D, Kumar V, Tyagi K. Comparative analysis of the two suborders of Thysanoptera and characterization of the complete mitochondrial genome of Thrips parvispinus. *Arch Insect Biochem Physiol*. (2023) 2(114):1–15. doi: 10.1002/arch.22010

30. Li C, Gao Y, Wang D, Dang L. The extraordinary rearrangement of mitochondrial genome of the wheat pest, Aptinothrips stylifer and the mitochondrial phylogeny of Thripidae (Thysanoptera). *Arch Insect Biochem Physiol*. (2024) 115: e22086. doi: 10.1002/arch.22086

31. Wolstenholme DR. Genetic novelties in mitochondrial genomes of multicellular animals. *Curr Opin Genet Dev*. (1992) 2:918–25. doi: 10.1016/S0959-437X(05)80116-9

32. Cameron SL. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annu Rev entomology*. (2014) 59:95–117. doi: 10.1146/annurev-ento-011613-162007

33. Krzywinski J, Grushko OG, Besansky NJ. Analysis of the complete mitochondrial DNA from Anopheles funestus: an improved dipteran mitochondrial genome annotation and a temporal dimension of mosquito evolution. *Mol Phylogenet Evol*. (2006) 39:417–23. doi: 10.1016/j.ympev.2006.01.006

34. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. (2018) 34:i884–90. doi: 10.1093/bioinformatics/bty560

35. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*. (2009) 25:1754–60. doi: 10.1093/bioinformatics/btp324

36. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. *Bioinformatics*. (2009) 25:2078–9. doi: 10.1093/bioinformatics/btp352

37. Li D, Liu CM, Luo R, Sadakane K, Lam TW. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*. (2015) 31:1674–6. doi: 10.1093/bioinformatics/btv033

38. Meng G, Li Y, Yang C, Liu S. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Res*. (2019) 47:e63–3. doi: 10.1093/nar/gkz173

39. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. (2013) 29:1072–5. doi: 10.1093/bioinformatics/btt086

40. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. (2014) 30:2068–9. doi: 10.1093/bioinformatics/btu153

41. Grant JR, Enns E, Marinier E, Mandal A, Herman EK, Chen CY, et al. Proksee: in-depth characterization and visualization of bacterial genomes. *Nucleic Acids Res*. (2023) 51:W484–92. doi: 10.1093/nar/gkad326

42. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Mol Phylogenet Evol*. (2013) 69:313–9. doi: 10.1016/j.ympev.2012.08.023

43. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol*. (2021) 38:3022–7. doi: 10.1093/molbev/msab120

44. Perna NT, Kocher TD. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J Mol Evol*. (1995) 41:353–8. doi: 10.1007/BF00186547

45. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. (2013) 30:772–80. doi: 10.1093/molbev/mst010

46. Li KB. ClustalW-MPI: ClustalW analysis using distributed and parallel computing. *Bioinformatics*. (2003) 19:1585–6. doi: 10.1093/bioinformatics/btg192

47. Inoue T, Sakurai T. The phylogeny of thrips (Thysanoptera: Thripidae) based on partial sequences of cytochrome oxidase I, 28S ribosomal DNA and elongation factor-1 α and the association with vector competence of tospoviruses. *Appl entomology zoology*. (2007) 42:71–81. doi: 10.1303/aez.2007.71

48. Liu Q, He J, Song F, Tian L, Cai W, Li H. Positive correlation of the gene rearrangements and evolutionary rates in the mitochondrial genomes of thrips (Insecta: Thysanoptera). *Insects*. (2022) 13:585. doi: 10.3390/insects13070585

49. Kumar V, Tyagi K, Kundu S, Chakraborty R, Singha D, Chandra K. The first complete mitochondrial genome of marigold pest thrips, Neohydatothrips samayunkur (Sericothripinae) and comparative analysis. *Sci Rep*. (2019) 9:191. doi: 10.1038/s41598-018-37889-6

50. Soumia PS, Shirsat DV, Krishna R, Choudhary JS, Naaz N, P.a. and Singh M. Unfolding the mitochondrial genome structure of green semilooper (Chrysodeixis

acuta Walker): An emerging pest of onion (Allium cepa L.). *PloS One*. (2022) 17: e0273635. doi: 10.1371/journal.pone.0273635

51. Karuppaiah V, Gadge AS, Shirsat DV, Soumia PS, Mainkar P, Kumar S, et al. The complete mitochondrial genome of the Indian dammer bee, Tetragonula iridipennis, and the phylogenomics of Meliponini. *Front Ecol Evol*. (2023) 11:1171242. doi: 10.3389/fevo.2023.1171242

52. Tyagi K, Chakraborty R, Cameron SL, Sweet AD, Chandra K, Kumar V. Rearrangement and evolution of mitochondrial genomes in Thysanoptera (Insecta). *Sci Rep*. (2020) 10:695. doi: 10.1038/s41598-020-57705-4

53. Xue XF, Deng W, Qu SX, Hong XY, Shao R. The mitochondrial genomes of sarcoptiform mites: are any transfer RNA genes really lost? *BMC Genomics*. (2018) 19:1–11. doi: 10.1186/s12864-018-4868-6

54. Beckenbach AT, Joy JB. Evolution of the mitochondrial genomes of gall midges (Diptera: Cecidomyiidae): rearrangement and severe truncation of tRNA genes. *Genome Biol Evol*. (2009) 1:278–87. doi: 10.1093/gbe/evp027

55. Masta SE, Boore JL. Parallel evolution of truncated transfer RNA genes in arachnid mitochondrial genomes. *Mol Biol Evol*. (2008) 25:949–59. doi: 10.1093/molbev/msn051

56. Margam VM, Coates BS, Hellmich RL, Agunbiade T, Seufferheld MJ, Sun W, et al. Mitochondrial genome sequence and expression profiling for the legume pod borer Maruca vitrata (Lepidoptera: Crambidae). *PloS One*. (2011) 6:e16444. doi: 10.1016/j.ijbiomac.2018.11.017

57. Gao S, Ren Y, Sun Y, Wu Z, Ruan J, He B, et al. PacBio full-length transcriptome profiling of insect mitochondrial gene expression. *RNA Biol*. (2016) 13:820–5. doi: 10.1080/15476286.2016.1197481

58. Ma Y, Zheng BY, Zhu JC, van Achterberg C, Tang P, Chen XX. The first two mitochondrial genomes of wood wasps (Hymenoptera: Symphyta): Novel gene rearrangements and higher-level phylogeny of the basal hymenopterans. *Int J Biol macromolecules*. (2019) 123:1189–96. doi: 10.1016/j.ijbiomac.2018.11.017

59. Mound LA. (2002). The Thrips and Frankliniella genus groups: the phylogenetic significance of ctenidia, in: *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, pp. 379–86. Australian National Insect Collection, Canberra, Australia.

60. Pandi A, Malaichamy K, Samuel KJ, Selvaraj D, Kasivelu G, Kolanthasamy E. Unveiling thrips morphology: A comparative analysis of microscopy-based ultrastructural, morphological, and molecular characterization of Thrips tabaci and Thrips parvispinus in onion. . *Microscopy Res technique*. (2024) 87:3003–15. doi: 10.1002/jemt.24653

61. Singh D, Kabiraj D, Sharma P, Chetia H, Mosahari PV, Neog K, et al. The mitochondrial genome of Muga silkworm (Antheraea assamensis) and its comparative analysis with other lepidopteran insects. *PloS One*. (2017) 12:e0188077. doi: 10.1371/journal.pone.0188077

62. Jiang SD, Wang L, Wang L, Sun J, Wang JJ, Wei DD. Mitochondrial coding genes mediate insecticide tolerance in the oriental fruit fly, Bactrocera dorsalis (Hendel). *Pesticide Biochem Physiol*. (2024) 199:105763. doi: 10.1016/j.pestbp.2023.105763

63. Guven C, Sevgiler Y, Taskin E. Pyrethroid insecticides as the mitochondrial dysfunction inducers. *Mitochondrial Dis*. (2018) 293:293–322. doi: 10.5772/intechopen.80283

64. Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L. Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: a review. *Insect Biochem Mol Biol*. (2010) 40:563–72. doi: 10.1016/j.ibmb.2010.05.008

65. Fotoukkiaii SM, Mermans C, Wybouw N, Van Leeuwen T. Resistance risk assessment of the novel complex II inhibitor pyflubumide in the polyphagous pest Tetranychus urticae. *J Pest Sci*. (2020) 93:1085–96. doi: 10.1007/s10340-020-01213-x

66. Hampl V, Pavlícek A, Flegr J. Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. *Int J systematic evolutionary Microbiol*. (2001) 51:731–5. doi: 10.1099/00207713-51-3-731