Original Article

STRUCTURAL VARIATIONS IN COMPLETE MITOGENOME OF STINGLESS BEES SERVE AS AUXILIARY MOLECULAR MARKERS FOR GENERIC SYSTEMATICS

Xinlan Chen[1] ORCID: 0000-0001-9294-425X
Yuxin Yao[2,3] ORCID: 0009-0006-3738-9752
Luo Zha[1] ORCID: 0009-0006-6076-1671
Jinshan Xu[2,3] ORCID: 0000-0002-4963-3410
Wenfeng Wang[1,4]* ORCID: 0009-0009-9027-936X

[1]Agricultural Research Institute, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, China
[2]Key Laboratory of Conservation and Utilization of Pollinator Insect of the upper reaches of the Yangtze River, Ministry of Agriculture and Rural Affairs, Chongqing 401331, Chongqing, China
[3]College of life sciences, Chongqing Normal University, Chongqing, China
[4]Agricultural Resources and Environment Institute, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, China

Received:20 July 2025; accepted: 14 January 2026
*corresponding author: 13989990522@163.com

Abstract

For this study, mitochondrial-level research was conducted into the stingless bee species *Lepidotrigona arcifera* in order to assemble the complete mitogenome sequence of *L. arcifera*, analyze its structural characteristics, and provide a more reliable and valuable taxonomic basis for resolving the complex phylogeny of *Meliponini* tribe. We obtained the complete mitogenome of *L. arcifera*, spanning 15,260 bp in length and comprising thirteen protein-coding genes (PCGs), two ribosomal RNAs (rRNAs), twenty-two transfer RNAs (tRNAs), and a control region (D-loop). Phylogenetic analysis showed that *L. arcifera* has the closest genetic relationship with *Lepidotrigona flavibasis* (genus *Lepidotrigona*), followed by *Lepidotrigona terminata* (genus *Lepidotrigona*), and then *Tetragonula pagdeni* (genus *Tetragonula*), consistent with our previous findings. We further conducted a comparative analysis of structural variations across mitogenomes, revealing significant divergence in gene order and transcriptional orientation. The thirty-eight mitochondrial genes were categorized into four blocks (I-IV) and the D-loop. An inversion within Block IV distinguished *L. arcifera* from its closest relative *L. flavibasis*. Conserved block order was highly consistent within *Melipona*. Notably, *Frieseomelitta varia* (genus *Frieseomelitta*) and *Tetragonisca angustula* (genus *Tetragonisca*) formed a distinct clade and shared an identical block arrangement, which differed from other species. This study presents the first complete mitogenome sequence of *L. arcifera*, enriching the genomic resources for this taxon. More importantly, it reveals extensive mitochondrial gene rearrangements in stingless bees, suggesting that such structural variations represent valuable auxiliary molecular markers for stingless bee species classification.

Keywords: classification, *Lepidotrigona arcifera*, mitogenome, molecular marker, phylogeny

INTRODUCTION

Contemporary hymenopteran research reveals a significant imbalance in taxonomic focus. While extensive knowledge exists on honeybees of such significant economic importance as *Apis mellifera* and *Apis cerana*, the highly eusocial stingless bees (Apidae: Meliponini), comprising over 600 species with complex taxonomy, exhibit substantial gaps in our understanding of their basic biological characteristics and taxonomic relationships (Rasmussen & Cameron, 2007; Attasopa et al., 2018). Stingless bees (Hymenoptera: Apidae: Meliponini) constitute a unique group of pollinating insects, and serve as primary pollinators for >80% of flowering plant species in tropical rainforests,

playing a vital role in maintaining plant genetic diversity (Silva et al., 2024; Patel et al., 2025).

This monophyletic tribe, encompassing ~45 genera and >600 described species, is characterized by intricate phylogenetic relationships, particularly among subgenera and species (Silva et al., 2024), primarily involving the species-rich genus groups *Trigona* and *Melipona*, alongside others like *Meliponula*, *Hypotrigona*, *Paratrigona, Plebeia* and *Trigonisca* (Klakasikorn et al., 2005). Current stingless bee taxonomy relies heavily on morphological characters (Rathor et al., 2013; Nidup, 2021). However, challenges arise from their small size, subtle interspecific morphological differences, and extensive overlap of key diagnostic features (e.g., pilosity, coloration, sculpturing, venation) across closely related species and even between genera (Rasmussen, 2013; Vijayakumar & Jayaraj, 2013; Tej et al., 2017), and this significantly impedes the accurate species delimitation, phylogenetic analysis, and diversity assessment of stingless bees globally.

Molecular data, particularly complete mitogenomes rich in phylogenetic information, remain extremely scarce (Oliveira et al., 2020; Engel et al., 2022). Of the hundreds of described stingless bee species, fewer than twenty possess publicly available complete mitogenomes in GenBank. This dearth of molecular data hinders the use of reliable genetic markers for verifying morphology, detecting cryptic species, and building robust phylogenies. Therefore, it is imperative to significantly augment the stingless bee molecular database, especially with mitogenomic data, to compensate for the limitations inherent in morphological approaches. While prior studies, e.g., Freitas et al. (2020) on *Frieseomelitta varia* mtDNA; Ferrari et al. (2024) on *Tetragonisca angustula* genomes; Li et al. (2024) on inverted repeats provide valuable insights, genomic data for taxa within some genera and smaller subgenera, including *Lepidotrigona*, are still scarce (Vijayakumar, 2014).

We identified the stingless bee *L. arcifera* (Hymenoptera: Meliponini) in Mêdog, Tibet, China (Cockerell, 1929; Yao et al., 2024). Currently known only from Tibet and Tuensang Village, India, this species exhibits a restricted distribution, which may reflect such unique biological traits as foraging behavior, nesting preferences, and environmental adaptation. Previous work which performed a preliminary assessment of its phylogenetic position within Apidae using partial mitochondrial sequences (*16S rRNA, COI*) suggest a close affinity to *L. flavibasis* (Yao et al., 2024). Limitations of single-gene data persist. Through sequencing and analysis of the complete mitogenome of *L. arcifera*, combined with published Apidae mitogenomes (NCBI), this study inferred a robust phylogeny, analyzed structural variation, and investigated the patterns of mitogenome rearrangements in Meliponini. These findings enhance our understanding of stingless bee evolution and provide new potential molecular markers based on genomic architecture for their systematics.

MATERIAL AND METHODS

Sample collection and DNA extraction

Stingless bee specimens (*L. arcifera*) were collected from Mêdog County, Tibet Autonomous Region, southwestern China (29°29’54.41”N 95°27’39.18”E) (Yao et al., 2024). Collected specimens were preserved in 75% ethanol and stored at 4°C. Total genomic DNA was isolated from thoracic muscle tissue with the use of the cetyltrimethylammonium bromide (CTAB) protocol and stored at -20°C until further analysis (Shi et al., 2020).

Mitogenome sequencing and assembly

Whole-genome sequencing of *L. arcifera* genomic DNA libraries was conducted on an Illumina HiSeq platform at Novogene Co., Ltd. (Beijing, China). Raw reads were quality-filtered using FASTX-Toolkit (v0.0.14) (Gordon & Hannon, 2010), discarding sequences with mean quality scores (Q) <20 and/or containing ambiguous bases (N). High-quality reads were assembled de novo using NOVOPlasty v4.3.1 with the *L. flavibasis* mitogenome (GenBank accession: MN747147.1) as a seed reference. Subsequent steps included genome annotation, validation, and visualization.

PCR and Sanger Sequencing for Gap Closure/ Validation: Gene-specific primers spanning

potential assembly gaps or ambiguous regions were designed via the NCBI Primer-BLAST tool (settings: e.g. product size 800-1500 bp, Tm ~60°C) and commercially synthesized by Sangon Biotech (Shanghai, China) (Tab. 1). Polymerase chain reaction (PCR) amplifications were performed in 40 µL reactions containing: 1 µL each of forward and reverse primers (10 µM), 300 ng template DNA, 1 µL Tks Gflex DNA Polymerase (1.25 U/ µL, Takara Bio), 20 µL 2x Gflex PCR Buffer, and nuclease-free water to a final volume of 40 µL. Thermal cycling conditions were 94°C for 3 min (pre-denaturation); 35 cycles of 98°C for 10 s (denaturation), 60°C for 15 s (annealing), 68°C for 5 min (extension); 68°C for 10 min (final extension). PCR products were size verified by 1% agarose gel electrophoresis and purified prior to bi-directional Sanger sequencing by Sangon Biotech. Sanger sequencing reads were assembled and base-called using SeqMan Pro v15.3.0 (DNASTAR Lasergene) (Tang et al., 2023) to correct and complete the mitogenome assembly. The final assembled mitogenome was annotated utilizing the MITOS2 web server (http://mitos2.bioinf.uni-leipzig.de/index.py) with default parameters for metazoans. Annotation was manually curated by comparison with the *L. flavibasis* mitogenome (MN747147.1) and visualized using Geneious Prime v2023.2.1 (Drummond et al., 2009) to generate a circular map. The complete mitogenome sequence of *L. arcifera* was deposited in GenBank under accession number PV599376.

Phylogenetic analysis

For phylogenetic reconstruction, the complete mitogenomes of seventeen bee species (representing Meliponini, Bombini, Apini, Megachilini) and *Drosophila melanogaster* (outgroup) were retrieved from GenBank (Accessions: *Lepidotrigona flavibasis* MN747147.1, *Lepidotrigona terminata* MN737481.1, *Melipona bicolor* AF466146.2, *Melipona flavolineata* PV243164.1, *Melipona mondury* PV243166.1, *Melipona rufiventris* PV243168.1, *Melipona scutellaris* KP202303, *Tetragonisca angustula* OR030859.2, *Tetragonula pagdeni* NC_066054.1, *Frieseomelitta varia* WNWW01002174.1, *Megachile sculpturalis* KT223644.1, *Bombini longipennis* NC_057952.1, *Apis mellifera scutellata* KJ601784.1, *Apis laboriosa* NC_036155.2, *Apis florea* KC170303.1, *Apis dorsata* NC_037709.1, *Drosophila melanogaster* NC_024511.2). Prior to alignment, gene order and strand orientation for all mitogenomes were standardized to match that of *L. arcifera* using manual curation. Nucleotide sequences of the entire mitogenomes were aligned using ClustalW as implemented in MEGA11 (Tamura et al., 2021). Phylogenetic trees were inferred using both Maximum Likelihood (ML) and Neighbor-Joining (NJ) methods within MEGA11. ML analysis was performed with 1000 bootstrap replicates. NJ analysis used p-distance with 1000 bootstrap replicates. Additionally, the thirteen PCGs were extracted from all eighteen mitogenomes, concatenated, and aligned. Phylogenetic analyses were performed identically on this concatenated

Table 1.

Primers used in this study

Primers	Nucleotide sequence (5'—3')
SL-1-F	GGGTATGAGCCTAATAGCTTAACTTAGC
SL-1-R	GCTATATCAGGTGAACCAAGTATAAG
SL-2-F	GCAAGTTTGAAAAAATTAATAGAATTAATTAATT
SL-2-R	GTATTGGAATAATAGTTCATACAATTTC
SL-3-F	AGGAATACCTCGTCGATACTCTGATTATCCTG
SL-3-R	GGTAAAAACTTACAAGTGTGTTAGAATAATAGGG
SL-4-F	GCTGCATTTGTAGTAATCATACTTTTCATATTTTCAATAATTG
SL-4-R	CCAATAGCTATAATAGCTCCAACTCCAGTGTCTTC
SL-5-F	CCTTGTTTCTTAGGTTCAGTTTTATAGTATTTAAAAATTCATG
SL-5-R	CAATACTCAGATCTTGAATAAGTAATATAAATTGCTGGATTG

dataset. Linear schematic representations depicting the arrangement of all thirty-eight genes were generated for comparative analysis.

RESULTS

Analysis of mitogenome structure and annotation

The mitogenome of *L. arcifera* (Fig. 1) comprises a circular double-stranded DNA molecule spanning 15,260 bp, encoding thirty-eight genes: thirteen protein-coding genes (PCGs: *COX1–3*, *ND1–6*, *ND4L*, *CYTB*, *ATP6*, *ATP8*), twenty-two transfer RNAs (tRNAs), two ribosomal RNAs (*16S* and *12S rRNA*), and a non-coding control region (D-loop) (Françoso et al., 2023). Nucleotide composition analysis revealed a pronounced AT bias (78.5%), with individual base frequencies of 37.9% A, 40.5% T, 11.0% G, and 10.6% C. The D-loop exhibited the highest AT content (84.1%) among all genomic regions. Full annotation details are provided in Tab. 2.

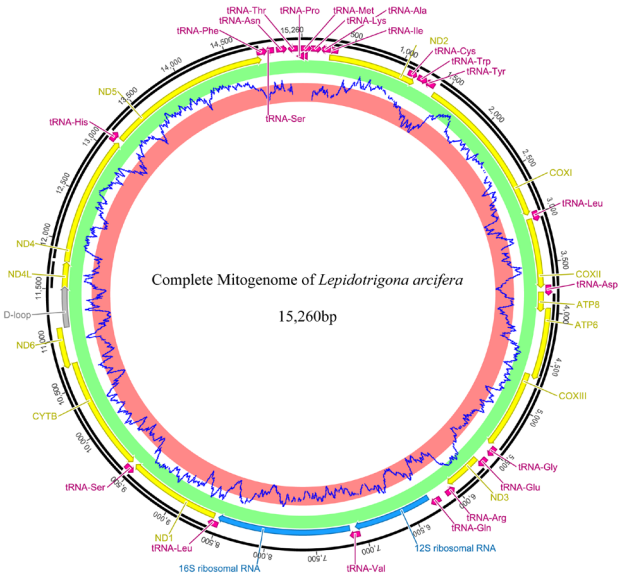


Fig. 1. Mitogenome mapping of *Lepidotrigona arcifera.*

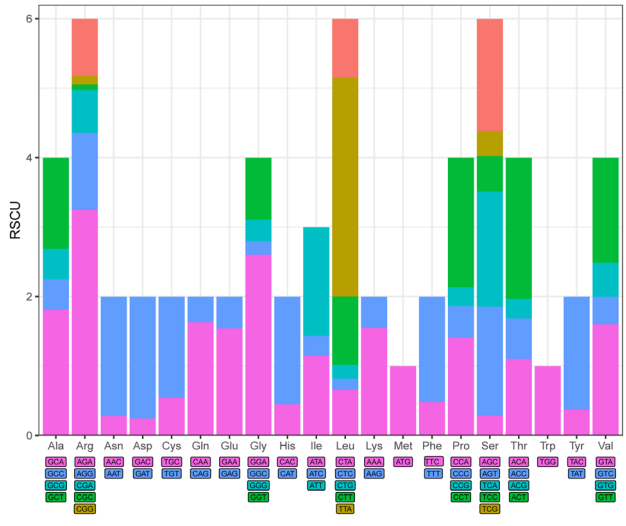


Fig 2. Codon bias statistics for 13PCGs in *L. arcifera* mitogenome, the length of the bar graph represents the cumulative score of all codons for each amino acid.

Codon bias analysis of coding genes

The thirteen PCGs collectively spanned 10,782 bp. Relative synonymous codon usage (RSCU) analysis demonstrated pervasive codon usage bias across all PCGs (RSCU>1; Fig. 2). NNA-type codons predominated (40.64% of total codons), indicating a strong preference for adenine at the third codon position. This pattern aligned closely with that observed in *L. flavibasis* (Wang et al., 2021).

Phylogenetic relationship

Phylogenetic reconstruction using *D. melanogaster* as the outgroup placed *L. arcifera* and *L. flavibasis* as sister taxa. These species formed a clade with *L. terminata* and *T. pagdeni*, which subsequently clustered with *Melipona* spp. to comprise a monophyletic stingless bee lineage (Fig. 3A). Stingless bees exhibited a closer affinity to *B. longipennis* (Bombini) than to *Apis* (Apini). Concordant topology was recovered from analyses of the thirteen concatenated PCGs (Fig. 3B). These results corroborate with our prior findings and robustly support the taxonomic placement of *L. arcifera*.

Structural variation of Mitogenome in stingless bees

There are many gene rearrangements in the mitogenomes among stingless bee species. To have a clearer understanding of the evolutionary classification of stingless bee species, weconductedin-depthresearchonthemitogenome structures of eleven stingless bee species including *L. arcifera*. All genes in the mitogenome of stingless bees are divided into four conserved blocks (Block I, Block II, Block III, Block IV) and one D-loop. According to the gene coding

Table 2.

Annotation of the *Lepidotrigona arcifera* mitogenome

Name of gene	Full name and function	Start position	End position	Length/bp	Strand
tRNA-Pro	Transfer RNA for Proline	1	69	69	-
tRNA-Met	Transfer RNA for Methionine	39	105	67	-
tRNA-Lys	Transfer RNA for Lysine	135	203	69	+
tRNA-Ala	Transfer RNA for Alanine	233	298	66	-
tRNA-Ile	Transfer RNA for Isoleucine	311	377	67	-
ND2	NADH dehydrogenase subunit 2	434	1133	700	+
tRNA-Cys	Transfer RNA for Cysteine	1134	1199	66	+
tRNA-Trp	Transfer RNA for Tryptophan	1244	1309	66	+
tRNA-Tyr	Transfer RNA for Tyrosine	1325	1393	69	-
COX1	Cytochrome c oxidase subunit I	1461	3020	1560	+
tRNA-Leu	Transfer RNA for Leucine	3033	3098	66	+
COX2	Cytochrome c oxidase subunit II	3099	3773	675	+
tRNA-Asp	Transfer RNA for Aspartic acid	3782	3847	66	+
ATP8	ATP synthase F0 subunit 8	3848	4015	168	+
ATP6	ATP synthase F0 subunit 6	4015	4692	678	+
COX3	Cytochrome c oxidase subunit III	4696	5475	780	+
tRNA-Gly	Transfer RNA for Glycine	5505	5573	69	+
tRNA-Glu	Transfer RNA for Glutamic acid	5644	5713	70	+
ND3	NADH dehydrogenase subunit 3	5714	6067	354	+
tRNA-Arg	Transfer RNA for Arginine	6073	6138	66	-
tRNA-Gln	Transfer RNA for Glutamine	6249	6315	67	+
12S rRNA	12S ribosomal RNA	6349	7116	768	+
tRNA-Val	Transfer RNA for Valine	7116	7181	66	+
16S rRNA	16S ribosomal RNA	7198	8544	1347	+
tRNA-Leu	Transfer RNA for Leucine	8545	8613	69	+
ND1	NADH dehydrogenase subunit 1	8614	9540	927	+
tRNA-Ser	Transfer RNA for Serine	9539	9605	67	-
CYTB	Cytochrome b	9614	10726	1113	-
ND6	NADH dehydrogenase subunit 6	10762	11292	531	-
D-loop	A+T-rich region	11293	11545	253	+
ND4L	NADH dehydrogenase subunit 4L	11546	11803	258	+
ND4	NADH dehydrogenase subunit 4	11833	13137	1305	+
tRNA-His	Transfer RNA for Histidine	13149	13213	65	+
ND5	NADH dehydrogenase subunit 5	13214	14863	1650	+
tRNA-Phe	Transfer RNA for Phenylalanine	14865	14929	65	+
tRNA-Ser	Transfer RNA for Serine	14944	15001	58	-
tRNA-Asn	Transfer RNA for Asparagine	15061	15127	67	+
tRNA-Thr	Transfer RNA for Threonine	15172	15237	66	-

order, Block I includes *ND2*, *COX1*, *COX2*, *ATP8*, *ATP6*, *COX3*, *ND3*; Block II includes *12S rRNA*, *16S rRNA*, *ND1*; Block III includes *ND6*, *CYTB*; Block IV includes *ND4L*, *ND4*, *ND5* (Fig. 4). The mitochondrial gene arrangement within the genus *Melipona* all presents as Block I(+)-IV(-)-III(+)-II(-) (+/-) indicates positive strand/reverse complement strand, *F. varia* and *T. angustula* are Block I(+)-III(+)-II(+)-IV(+), *L. flavibasis*, *L. terminata* and *T. pagdeni* are Block I(+)-II(+)-III(-)-IV(-), and *L. arcifera* is Block I(+)-II(+)-III(-)-IV(+). The different arrangements

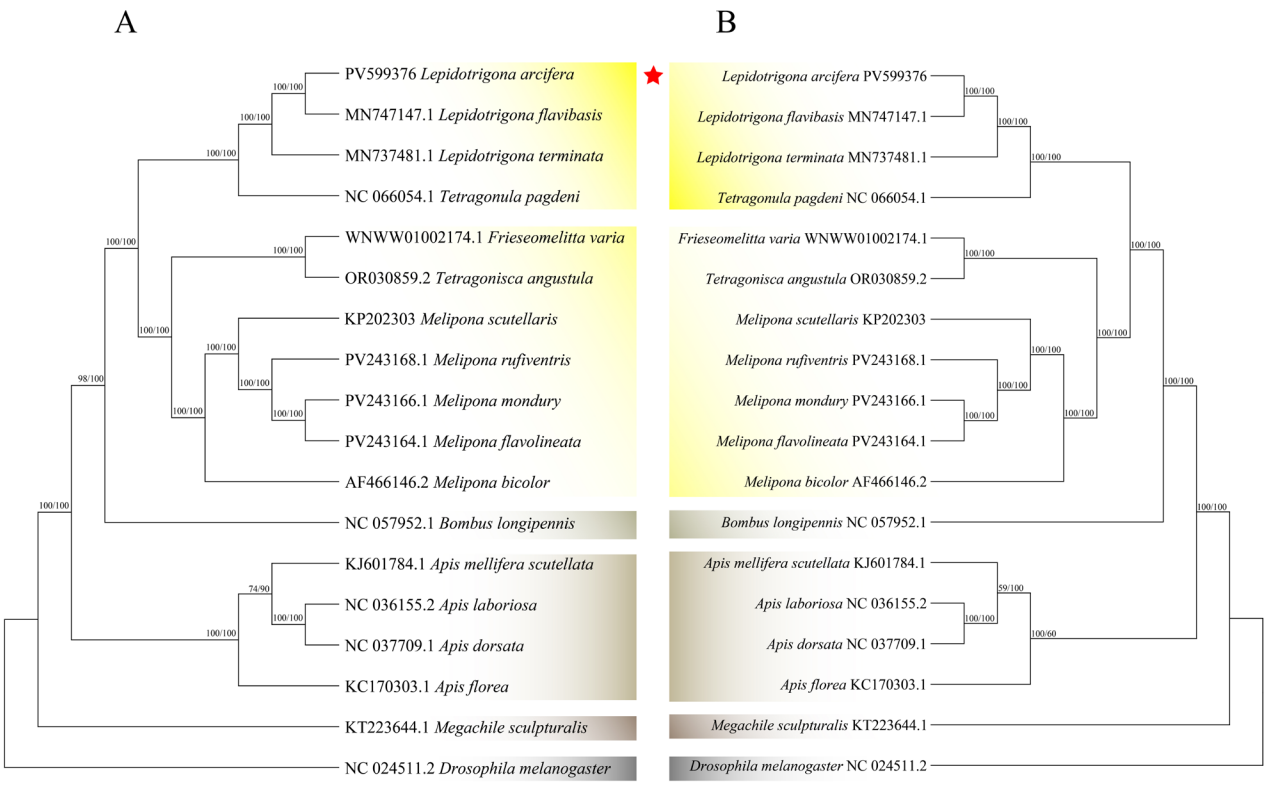


Fig 3. (A) Phylogenetic relationships based on full-length mitochondria, bootstrap values present in the above branch using neighbor-joining and maximum-likelihood methods, the species *Drosophila melanogaster* was used as the outgroup. (B) Phylogenetic relationships based on 13 PCGs.

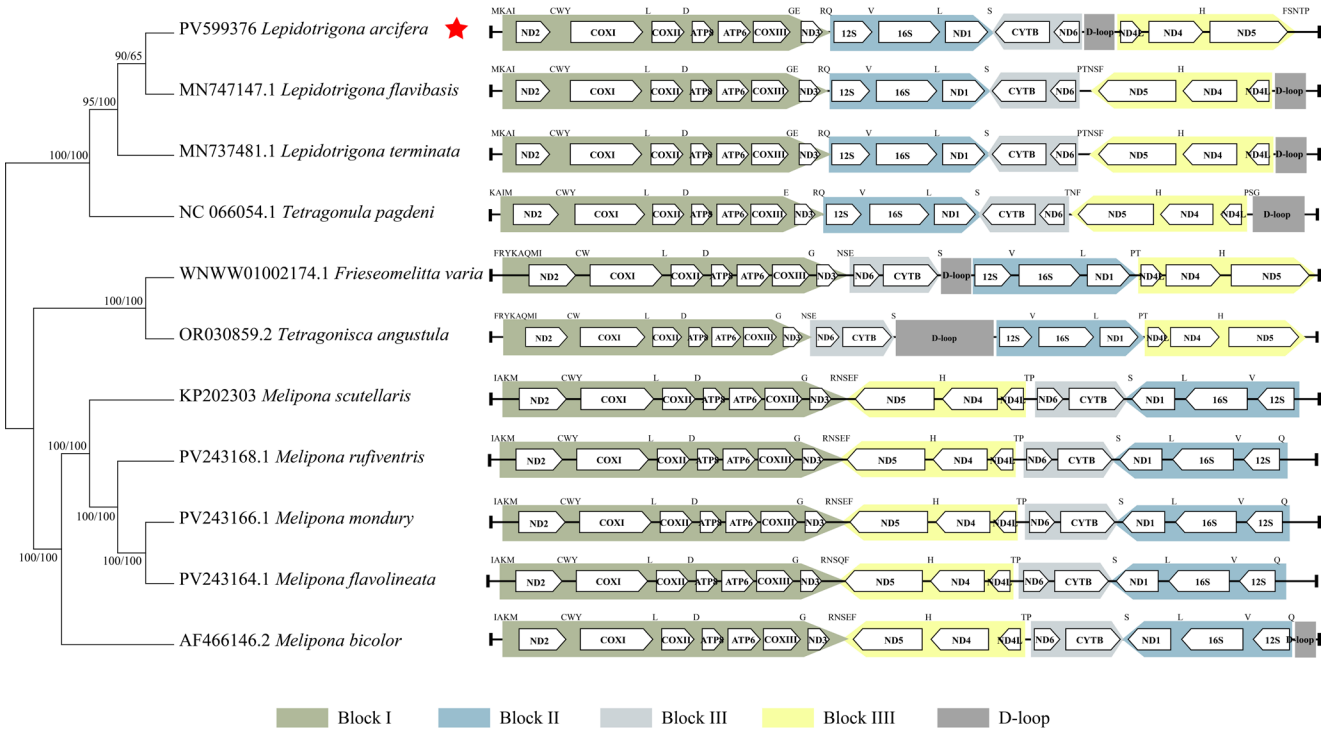


Fig 4. Schematic diagram of the arrangement of 37 genes in the mitogenome.

of the four conserved blocks can serve as references for identifying and distinguishing different genera within stingless bees, and the division of conserved blocks is shown in Fig. 4. Meanwhile, in the comparison of the mitogenome of *L. arcifera* with those of its closest evolutionary relatives *L. flavibasis* (MN747147.1), *L. terminata* (MN737481.1), and *T. pagdeni* (NC_066054.1), we found that there is an overall inversion of a sequence in the *L. arcifera* mitogenome. The coding order of this sequence in *L. arcifera* is D-loop-Block IV (*ND4L-ND4-ND5*), while in *L. flavibasis*, *L. terminata*, and *T. pagdeni*, it is Block IV (*ND5-ND4-ND4L*)-D-loop. The discovery of this rearrangement phenomenon provides a reference molecular marker for species identification within the genus *Lepidotrigona*. In addition, in *Lepidotrigona*, a tRNA unit of $tRNA^{Met}$-$tRNA^{Lys}$-$tRNA^{Ala}$-$tRNA^{Ile}$ is formed before Block I, while in *Melipona*, this unit is reversed to $tRNA^{Ile}$-$tRNA^{Ala}$-$tRNA^{Lys}$-$tRNA^{Met}$. The rearrangement of this tRNA unit can also serve as potential evidence for distinguishing different genera of stingless bees.

DISCUSSION

Within the family Apidae, the tribes Meliponini (stingless bees) and Apini (honey bees) represent the only two highly eusocial lineages. The Meliponini tribe encompasses over 600 described species classified into approximately forty-five genera. Despite significant contributions to the taxonomy of this tribe, its high biodiversity necessitates continued research. This study successfully assembled and reports the first complete mitogenome of *L. arcifera*, a 15,260 bp circular molecule. Its gene content (13 PCGs, 22 tRNAs, 2 rRNAs and one major non-coding control region) are consistent with other reported stingless bee mitogenomes (Wang et al., 2020; Wang et al., 2021).

Nucleotide composition analysis revealed a high AT content (78.5%) in the *L. arcifera* mitogenome, indicating a AT bias. Such compositional differences may suggest the existence of potential adaptive evolutionary characteristics among different geographical populations or species under environmental selection pressures (Yuan et al., 2024). Phylogenetic analysis confirmed that *L. arcifera* forms a close sister clade with *L. flavibasis*, supporting their close relationship within the stingless bees. This clade groups with other stingless bee species, and subsequently with *Melipona* species, forming a large stingless bee clade, consistent with our previous findings (Wang et al., 2022; Ferrari et al., 2024).

Through comparative analysis of the mitogenomes of multiple stingless bee species, we revealed the existence of complex gene rearrangement events: differences in the arrangement order and coding direction of different blocks can clearly distinguish different groups within the Meliponini. Block I maintains a high degree of conservation in position and coding direction in all analyzed mitogenomes of stingless bees, indicating that it may be the most stable sequence module in this lineage. In contrast, Block II, Block III, and Block IV show significant variations among different genera. This variation pattern may position them as promising molecular markers applicable to species identification and classification of the highly diverse group of stingless bees (Gonçalves et al., 2024). It is worth noting the phylogenetic analysis results, *Frieseomelitta* and *Tetragonisca* are traditionally regarded as subgenera of the genus *Trigona* (Michener, 1990). The phylogenetic tree constructed in this study shows that *F. varia* and *T. angustula* are distantly related to other stingless bee genera within the analyzed clade, additionally the gene arrangement orders of *F. varia* and *T. angustula* are highly similar and have fundamental differences from the other genera (Fig. 4). Moreover, recent studies have elevated them to the rank of distinct genera and confirmed this as a more appropriate classification (Engel et al., 2023; Li et al., 2024), which is consistent with and further corroborated by the findings of the present study. In addition, the unique evolutionary branch formed by *F. varia* and *T. angustula* may reflect the insufficiently recognized lineage differentiation history within the Meliponini, and their significant differences from other genus species emphasize the complexity of the taxonomic system of the Meliponini, which needs to be further clarified

with multi-dimensional evidence (Engel et al., 2018).

Compared with the Eastern honeybee (*Apis cerana*) and the Western honeybee (*Apis mellifera*), the mitogenome of stingless bees shows a higher evolutionary rate. This accelerated evolution may constitute the genetic basis for their adaptation to diverse ecological niches in tropical and subtropical regions (Cristiano et al., 2012; Silverio et al., 2014; Françoso et al., 2023). This study not only provides key molecular basis for the species identification of *L. arcifera* but, more importantly, supplements important molecular marker data based on the complete mitogenome for clarifying the phylogenetic relationships of the Meliponini.

However, this study still has limitations. The current data are only from a single geographical population, and future studies need to expand the geographical sampling range to evaluate the stability of the genetic structure and geographical variation patterns of this species. The mitogenome can only reflect maternal genetic history, so to comprehensively analyze the speciation and evolutionary process of stingless bees, subsequent studies need to integrate nuclear gene data (such as ITS sequences or microsatellite markers) to deeply explore the nuclear genome of stingless bees (Rasmussen et al., 2017). The analysis of positive selection or adaptive evolution of key functional genes (such as *ATP6*, *NADH* dehydrogenase series genes) still needs to be deepened to reveal the potential correlation mechanism between their functional variations and the ecological adaptability of stingless bees (Rozen et al., 2019; Xu et al., 2025).

In conclusion, this study effectively addresses a knowledge gap in stingless bee mitogenome data. It provides valuable molecular evidence and scientific reference for the global taxonomic delimitation of stingless bees, phylogenetic reconstruction, and the formulation of resource conservation strategies. Future research, building upon the mitogenome dataset established here, could focus on cross-species comparative genomics, fine-scale analysis of population genetic structure, and exploration of molecular mechanisms underlying ecological adaptation. Such efforts will deepen our understanding of stingless bee diversity and promote their comprehensive conservation and sustainable utilization.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Dr. Xiangyou Tang and Dr. Yaohui Li from Chongqing Normal University for their contributions to data processing and experimental guidance in this work. During the manuscript preparation, DeepSeek (https://chat.deepseek.com/) online language model was utilized for language polishing.

FUNDING

Supported by The earmarked fund for China Agriculture Research System (No.CARS-44); Science and Technology Program of Tibet Autonomous Region (No.XZ202102YD0020C); Creation & research team in College and Universities of Chongqing Municipal Education Commission (No.CXQT21013) and Chongqing Modern Agricultural Industry Technology System (COMAITS202416).

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships.

REFERENCES

Attasopa, K., Bänziger, H., Disayathanoowat, T., Packer, L. (2018). A new species of *Lepidotrigona* (Hymenoptera: Apidae) from Thailand with the description of males of *L. flavibasis* and *L. doipaensis* and comments on asymmetrical genitalia in bees. *Zootaxa, 4442*(1), 063-082.https://doi.org/10.11646/zootaxa.4442.1.3

Cockerell, T. D. A. (1929). LXX.-Descriptions and records of bees.-CXX. *Annals and Magazine of Natural History, 4*(24), 584-594. https://doi.org/10.1080/00222932908673101

Cristiano, M. P., Fernandes-Salomão, T. M.,

Yotoko, K. S. C. (2012). Nuclear mitochondrial DNA: an Achilles' heel of molecular systematics, phylogenetics, and phylogeographic studies of stingless bees. *Apidologie, 43*, 527-538. https://doi.org/10.1007/s13592-012-0122-4

Drummond, A., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R., ... Wilson, A. (2009). Geneious v4.7. http://www.geneious.com/

Engel, M. S., Kahono, S., Peggie, D. (2018). A key to the genera and subgenera of stingless bees in Indonesia (Hymenoptera: Apidae). *Treubia, 45,* 65-84. https://doi.org/10.14203/treubia.v45i0.3687

Engel, M. S., Nguyen, L. T. P., Tran, N. T., Truong, T. A., Motta, A. F. H. (2022). A new genus of minute stingless bees from Southeast Asia (Hymenoptera, Apidae). *ZooKeys,* (1089), 53-72. https://doi.org/10.3897/zookeys.1089.78000

Engel, M. S., Rasmussen, C., Ayala, R., de Oliveira, F. F. (2023). Stingless bee classification and biology (Hymenoptera, Apidae): a review, with an updated key to genera and subgenera. *ZooKeys, 1172*, 239-312. https://doi.org/10.3897/ zookeys.1172.104944

Ferrari, R. R., Ricardo, P. C., Dias, F. C., Araujo, N. D. S., Soares, D. O., Zhou, Q. S., ... Batista, T. M. (2024). The nuclear and mitochondrial genome assemblies of *Tetragonisca angustula* (Apidae: Meliponini), a tiny yet remarkable pollinator in the Neotropics. *BMC Genomics, 25*(1), 587. https://doi.org/10.1186/s12864-024-10502-z

Françoso, E., Zuntini, A. R., Ricardo, P. C., Santos, P. K. F., Araujo, N. D. S., Silva, J. P. N., ... Taylor, B. A. (2023). Rapid evolution, rearrangements and whole mitogenome duplication in the Australian stingless bees *Tetragonula* (Hymenoptera: Apidae): A steppingstone towards understanding mitochondrial function and evolution. *International Journal of Biological Macromolecules, 242(*1) 124568. https://doi.org/10.1016/j.ijbiomac.2023.124568

Freitas, F. C. D. P., Lourenço, A. P., Nunes, F. M. F., Paschoal, A. R., Abreu, F. C. P., Barbin, F. O., ... Silva, S. R. (2020). The nuclear and mitochondrial genomes of *Frieseomelitta varia* - a highly eusocial stingless bee (Meliponini) with a permanently sterile worker caste. *BMC Genomics, 21*(1), 386. https://doi.org/10.1186/s12864-020-06784-8

Gonçalves, L. T., Françoso, E., Deprá, M. (2024) Mitochondrial phylogenomics of bumblebees, *Bombus* (Hymenoptera: Apidae): a tale of structural variation, shifts in selection constraints, and tree discordance. *Zoological Journal of the Linnean Society, 202*(1). https://doi.org/10.1093/zoolinnean/zlad178

Gordon, A., & Hannon, G. J. (2010). Fastx-toolkit. FASTQ/A short-reads pre-processing tools (unpublished) http://hannonlab. cshl. edu/fastx_ toolkit.

Klakasikorn, A., Wongsiri, S., Deowanish, S., Duangphakdee, O. (2005). New record of stingless bees (Meliponini: *Trigona*) in Thailand. *Tropical Natural History, 5*(1), 1-7. https://doi.org/10.58837/tnh.5.1.102885

Li, Y. R., Wang, Z. W., Corlett, R. T., Yu, W. B. (2024). Comparative analyses of mitogenomes in the social bees with insights into evolution of long inverted repeats in the Meliponini. *Zoological Research, 45*(1), 160-175. https://doi.org/10.24272/j.issn.2095-8137.2023.169

Michener, C. D. (1990). Classification of the Apidae (Hymenoptera). *Mi. Paper, 54*(4), 75-164. https://digitalcommons.usu.edu/bee_lab_mi/153

Nidup, T. (2021). Report on the stingless bees of Bhutan (Hymenoptera: Apidae: Meliponini). *Journal of Threatened Taxa, 13*(5), 18344-18348. https://doi.org/10.11609/jot.4504.13.5.18344-18348

Oliveira, F. F. D., Silva, L. R. D. S., Zanella, F. C. V., Garcia, C. T., Pereira, H. L., Quaglierini, C., Pigozzo, C. M. (2020). A new species of *Ceratina* (*Ceratinula*) Moure, 1941, with notes on the taxonomy and distribution of *Ceratina* (*Ceratinula*) *manni* Cockerell, 1912, and an identification key for species of this subgenus known from Brazil (Hymenoptera, Apidae, Ceratinini). *ZooKeys,*

1006, 137-165. https://doi.org/10.3897/zookeys.1006.57599

Patel, Z. N., Dodiya, R. D., Pathan, N. P., Sai Thilak, K., Patel, P. S. (2025). Buzzing wonders: a comprehensive review of stingless bee biology and behaviour. *Journal of Agriculture and Ecology, 20*(2025), 1-6.https://doi.org/10.58628/JAE-2520-101

Rasmussen, C. (2013). Stingless bees (Hymenoptera: Apidae: Meliponini) of the Indian subcontinent: Diversity, taxonomy and current status of knowledge. *Zootaxa, 3647*(3), 401-428.https://doi.org/10.11646/zootaxa.3647.3.1

Rasmussen, C., & Cameron, S. A. (2007). A molecular phylogeny of the Old World stingless bees (Hymenoptera: Apidae: Meliponini) and the non-monophyly of the large genus *Trigona. Systematic Entomology, 32*(1), 26-39.https://doi.org/10.1111/j.1365-3113.2006.00362.x

Rasmussen, C., Thomas, J. C., Engel, M. S. (2017). A new genus of Eastern Hemisphere stingless bees (Hymenoptera: Apidae), with a key to the supraspecific groups of Indomalayan and Australasian meliponini. *American Museum Novitates*, *3888*, 1-33. DOI: 10.1206/3888.1

Rathor, V. S., Rasmussen, C., Saini, M. S. (2013). New record of the stingless bee *Tetragonula gressitti* from India (Hymenoptera: Apidae: Meliponini). *Journal of Melittology, 7,* 1-5. https://doi.org/10.17161/jom.v0i7.4454

Rozen, J. G., Quezada-Euán, J. J. G., Roubik, D. W., Smith, C. S. (2019). Immature Stages of Selected Meliponine Bees (Apoidea: Apidae). *American Museum Novitates, 2019*(3924), 1-28. https://doi.org/10.1206/3924.1

Shi, P., Zhou, J., Song, H. L., Wu, Y. J., Lan, L., Tang, X. Y., … Xu, J. X. (2020). Genomic analysis of Asian honeybee populations in China reveals evolutionary relationships and adaptation to abiotic stress. *Ecology and Evolution, 10*(23), 13427-13438.https://doi.org/10.1002/ece3.6946

Silva, G. R. D., Souza, I. G. D. B., Pereira, F. D. M., Souza, B. D. A., Lopes, M. T. D. R., Prosdocimi, F., … Diniz, F. M. (2024). The mitochondrial genome of *Melipona fasciculata* (Apidae, Meliponini): genome organization and comparative analyses, phylogenetic implications and divergence time estimations. *Biochemical Genetics*, *63*, 5312-5337. https://doi.org/10.1007/s10528-024-10991-3

Silverio, M. S., Rodovalho, V. D. R., Bonetti, A. M., Oliveira, G. C. D., Cuadros-Orellana, S., … Rodrigues Dos Santos, A. (2014). Preliminary characterization of mitochondrial genome of *Melipona scutellaris*, a Brazilian stingless bee. *BioMed Research International, 2014*, 1-6, Article ID: 927546. https://doi.org/10.1155/2014/927546

Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022-3027.https://doi.org/10.1093/molbev/msab120

Tang, X. Y., Yao, Y. X., Li, Y. H., Song, H. L., Luo, R., Shi, P., … Xv, J. S. (2023). Comparison of the mitochondrial genomes of three geographical strains of *Apis laboriosa* indicates high genetic diversity in the black giant honeybee (Hymenoptera: Apidae). *Ecology and Evolution, 13*(2), e9782. https://doi.org/10.1002/ece3.9782

Tej, M. K., Srinivasan, M. R., Vijayakumar, K., Natarajan, N., Kumar, S. M. (2017). Morphometry analysis of stingless bee *Tetragonula iridipennis* Smith (1854). *International Journal of Current Microbiology Applied Sciences, 6*(10), 2963-2970. https://doi.org/10.20546/ijcmas.2017.610.350

Vijayakumar, K. (2014). Nest and colony characters of *Trigona* (*Lepidotrigona*) *ventralis* var. *arcifera* Cockerell from North East India. *Asian Journal of Conservation Biology, 3*(1), 90-93.

Vijayakumar, K., & Jayaraj, R. (2013). Geometric morphometry analysis of three species of stingless bees in India. *International Journal for Life Sciences and Educational Research, 1*(2), 91-95.

Wang, C. Y., Yang, P. L., Zhao, M., Xu, H. L., Liu, L. N., Feng, Y., Wang, S. J. (2022). Unusual mitochondrial

tRNA rearrangements in stingless bee *Tetragonula pagdeni* and phylogenetic analysis. *Entomological Science, 25*(4), e12526. https://doi.org/10.1111/ens.12526

Wang, C. Y., Zhao, M., Wang, S. J., Xu, H. L., Yang, Y. M., Liu, L. N., Feng, Y. (2021). The complete mitochondrial genome of *Lepidotrigona flavibasis* (Hymenoptera: Meliponini) and high gene rearrangement in *Lepidotrigona* mitogenomes. *Journal of Insect Science, 21*(3), 1-7. https://doi.org/10.1093/jisesa/ieab038

Wang, C. Y., Zhao, M., Xu, H. L., Zhang, F. L., Zhong, Y. H., Feng, Y., Wang, S.. J. (2020). Complete mitochondrial genome of the stingless bee *Lepidotrigona terminata* (Hymenoptera: Meliponinae) and phylogenetic analysis. *Mitochondrial DNA Part B, 5*(1), 752-753. https://doi.org/10.1080/23802359.2020.1715298

Xu, Y. D., Zheng, X., Tang, B. Y., Yang, X. R., Vit, P., Popova, M., … Wang, K. (2025). Mannitol: A novel chemical marker in stingless bee honey. *Food Chemistry, 472,* 142967. https://doi.org/10.1016/j.foodchem.2025.142967

Yao, Y. X., Tang, X. Y., Li, Y. H., Song, H. L., Jiang, Y., Chen, X. L., … Xv, J. S. (2024). Taxonomic status of stingless bee *Lepidotrigona arcifera* (Hymenoptera: Meliponini) from the Southwest China-Tibetan Plateau. *Journal of Apicultural Science, 68*(2), 133-142. https://doi.org/10.2478/jas-2024-0008

Yuan, B., He, G., Dong, W. (2024). The evolutionary characterization of Gamasida based on mitochondrial genes codon usage pattern. *Parasitology Research, 123*(30), 29-30. https://doi.org/10.1007/s00436-023-08019-7.