Two new species of the leafhopper genus *Arboridia* (Hemiptera, Cicadellidae, Typhlocybinae, Erythroneurini) from southern China based on morphology and complete mitogenomes.

Ni Zhang[1,2], Jinqiu Wang[1,2], Tianyi Pu[1,2], Can Li[3] and Yuehua Song[1,2]*

[1] School of Karst Science, Guizhou Normal University, Guiyang 550001, China

[2] State Engineering Technology Institute for Karst Desertification Control, Guiyang 550001, China

[3] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region/ Guizhou Provincial Engineering Research Center for Biological Resources Protection and Efficient Utilization of the Mountainous Region, Guiyang University, Guiyang, 550005, China;

* Corresponding authors
Yuehua Song, songyuehua@163.com

Abstract: In the present work, morphology and mitogenomes are utilized to resolve the relationship between four erythroneurine leafhoppers (Hemiptera, Cicadellidae, Typhlocybinae, Erythroneurini): *Arboridia* (*Arboridia*) *rongchangensis* sp. nov., *Thaia* (*Thaia*) *jiulongensis* sp. nov., *Mitjaevia bifurcata* and *Mitjaevia diana*, the two new species are described and illustrated. The mitochondrial gene sequences of these four species were determined to update the mitochondrial genome database of Erythroneurini. The mitochondrial genomes of four species shared high parallelism in nucleotide composition, base composition and gene order, comprising 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs) and a AT control region, which was consistent with majority of species in Cicadellidae; all genes revealed common trait of a positive AT skew and negative GC skew. The mitogenomes of four species were ultra-conservative in structure, and which is analogous to that of others in size and A+T content. Phylogenetic trees based on the mitogenome data of these species and another 24 species were built employing the maximum likelihood and Bayesian inference methods. The results indicated that the four species belong to the tribe

Deleted: Erythroneurini

Deleted: combined with other two Erythroneurine species and their phylogenetic status

Deleted: Erythroneurine leafhoppers (Hemiptera, Cicadellidae, Typhlocybinae, Erythroneurini) are a significant economic pest, causing heavy economic losses to both agriculture and forestry. The combination of morphological and molecular characteristics is valuable to species delimitation and the analysis of their phylogenetic relationships. …

Deleted: the

Deleted: were analyzed

Deleted: the

Moved (insertion) [1]

Commented [MW1]: Add author and date

Commented [MW2]: ditto

Deleted: leafhopper species. The two new species, *Arboridia* (*Arboridia*) *rongchangensis* sp. nov. and *Thaia* (*Thaia*) *jiulongensis* sp. nov.

Deleted: Meanwhile,

Moved up [1]: *Arboridia* (*Arboridia*) *rongchangensis* sp. nov., *Thaia* (*Thaia*) *jiulongensis* sp. nov., *Mitjaevia bifurcata* and *Mitjaevia diana*

Deleted: including *Arboridia* (*Arboridia*) *rongchangensis* sp. nov., *Thaia* (*Thaia*) *jiulongensis* sp. nov., *Mitjaevia bifurcata* and *Mitjaevia diana*

Erythroneurini, *M. diana* is the sister-group relationship of *M. protuberanta* + *M. bifurcata*. The two species *Arboridia* (*Arboridia*) *rongchangensis* sp. nov. and *Thaia* (*Thaia*) *jiulongensis* sp. nov. also have a relatively close genetic relationship with the genus *Mitjaevia*.

Key words: New species; Morphology; Mitochondrial genome; Phylogenetic analysis; Karst

Introduction

The tribe Erythroneurini Young, 1952, the largest tribe in the leafhopper subfamily Typhlocybinae, (Hemiptera, Cicadellidae), comprising 204 genera and nearly 2000 described species (*Young, 1952*; *Dmitriev, 2003*; *Song & Li, 2016*; *Song & Li, 2017*), and is widely distributed in all major zoogeographic regions of the world (*Chen et al., 2021a*). As plant sap sucking insects they are among the main pests of agriculture, including fruit trees and vegetables, and their small size makes them difficult to detect and identify. Damage to plants is by egg laying and as virus vectors of plant pathogens (*Womack & Schuster, 1986*; *Bellota, 2011*; *Bosco et al., 2007*). Moreover, erythroneurine species have adapted to various habitats and plants such as trees, rocks, grasslands, sandy substrates, and bushy areas etc. (*Morris, 1971*; *Roddee et al., 2018*). The species of Erythroneurini have been divided and classified by researchers in different ways, as a result of high morphological diversity and wide geographical distributions.

China is the country with the most widely distributed, fully developed and most complete types of karst landforms in the world, which are mainly concentrated in carbonate outcropping areas, of which Guangxi, Guizhou, and eastern Yunnan account for the largest area (*Xiong et al., 2008*). Guizhou is an important part of the Yunnan-Guizhou Plateau and is believed to be the most well-developed representative of karst areas. The terrain is violently undulating, the types of landforms are diverse, and the composition of surface material and soil types is complex. Additionally, the

Deleted: is

Deleted: which belongs to

Deleted: , Typhlocybinae,

Deleted: the six

Deleted: They are herbivorous

Deleted: and

Deleted: ,

Deleted: , and flowers

Deleted: . They pose a huge threat to the development of agricultural and forestry production, since the

Deleted: that is not easy to discover

Deleted: There are several ways to d

Deleted: through feeding, spawning, excretion and the spread of the

Deleted: E

Deleted: occupy assorted morphological and ethological adaptations to omnifarious surroundings, containing

Deleted: , and so on

Deleted: variability

climate of this area is warm and humid, with small annual temperature changes, warm in winter and cool in summer (*Zhu et al., 2020*), resulting in high biodiversity (*Luo et al., 2016; Zhu, 2007*). Many new species of Erythroneurini were discovered in karst regions from Guizhou (*Chen et al., 2020; Song et al., 2011; Zhang et al., 2021*).

Phylogenetic relationships of major lineages of Cicadellidae have been researched for many years (*Chen & Li, 2014; Wang et al., 2020a; Wang et al., 2020b*). More recently, diverse molecular markers have been applied to perform phylogenetic inferences of Hemiptera, which consist of shape characteristic, mitochondrial genes, nuclear genes and a combination of them, together with transcriptomes on the basis of next-generation sequencing (*Almeida et al., 2009; Wang et al., 2010; Yao et al., 2021*). With the purpose of confirming the results of traditional classification of Eurythroneurini we also use molecular markers. Based on morphological and molecular data, Erythroneurini has been divided into 204 genera (*Dmitriev, 2003*). However, in most instances, short time intervals between speciation events generated incongruous divergence in morphological features and molecular markers (*Chen et al., 2021b*).

The relationships among the multiple species of Cicadellidae were established by means of morphological characters, and a few nuclear genes and mitochondrial sequences (*Longo et al., 2017; Jiang et al., 2021*). However, despite the tendency to expand genome coverage, the number of specimens that can be collected is relatively limited, and existing species were chosen to conduct genetic sequencing, as it is impossible to establish a relatively complete molecular identification of the family. Therefore, in our work, the mitochondrial genomes of two new species and two known species (*Mitjaevia bifurcata*, *Mitjaevia diana*) were picked via Sequencing Technology to provide a comprehensive comparative analysis of mitochondrial gene structure (*Luo et al., 2021; Distant &*

Deleted: . Karst ecosystems hold

Deleted: (plants, insects, birds, snails and bats), except that singular and goodliness natural landscape

Deleted: With the purpose of confirming the results of traditional classification, molecular markers were used to further study the phylogenetic relationship of leafhoppers. A phylogenetic development analysis for Erythroneurini is necessary to understand the taxonomic and evolutionary relationships among Cicadellidae and also conducive to phylogenic inference of Hemiptera (*Chen et al., 2021b*).

Deleted: over score of

Deleted: D

Deleted: was

Deleted: Therefore, except for the complicated morphological classification of species, molecular technology has also become an important means to explore phylogenetic analyses among species in recent years.

Deleted: acked

Rhynchota, 1918). Phylogenetic trees based on the mitochondrial genomes of *A.* (*A.*) *rongchangensis* sp. nov. and *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* and another 24 species were built adopting the Bayesian inference and maximum likelihood methods. This research will enrich the mitochondrial gene bases of the erythroneurine leafhoppers and improve the accuracy of the traditional classification.

Commented [MW3]: Why is this here?

Materials and methods

Leafhopper Collections and Species Identification Based on the Morphology

The species of leafhopper are collected according to Table 1. The specimens were preserved in absolute ethanol. Images of the appearance and genitalia of species were taken by a KEYENCE VHX-5000 digital microscope. Male/female specimens were identified under a stereoscope, and the whole abdomen of the specimens was separated and moistened in a hot 10% NaOH solution. Afterward, the abdomen was washed with ordinary water, blotted up with qualitative filter paper, and transferred to a clean glass slide with a drop of glycerin. Genital dissections were dissected in glycerin to inhibit parts from drying out. Then, they were viewed and plotted by way of Olympus SZX16 and BX53 microscopes. The remaining specimen was stored in 95% ethanol and put in a refrigerator at −20°C. The analyzed specimens were examined using Olympus SZX16 dissecting microscope and Olympus BX53 stereoscopic microscopes respectively and identified by Prof. Yuehua Song. All specimens inspected are reserved in the School of Karst Science, Guizhou Normal University, China (GZNU).

DNA Extraction, Mitogenome Sequencing and Assembly

Extraction of DNA originated from the whole body removing the abdomen and wings. The bodies were incubated at 56 °C for 6 h for complete lysis and total genomic DNA was eluted in 50

μL double-distilled water (ddH_2O), and the remaining other steps were performed according to the manufacturer's protocol. Genomic DNA was stored at −20 °C. The whole mitochondrial genomes of *A.* (*A.*) *rongchangensis* sp. nov. and *T.* (*T.*) *jiulongensis* sp. nov. were sequenced at Berry Genomics (Beijing, China) by an Illumina Novaseq 6000 platform (Illumina, Alameda, CA, USA) using 150 bp paired-end reads. Firstly, the obtained sequence reads were filtered following Zhou et al. (*Zhou et al., 2013*), the remaining high-quality reads were assembled by an iterative De Bruijin graph de novo assembler, the IDBA-UD toolkit, with a similarity threshold of 98%, and k values of 40 and 160 bp (*Peng et al., 2012*). The mitogenome was initially assembled by Geneious Prime v 2021.1.1, and then manually proofread based on sequencing peak figures.

The complete mitochondrial genomes of *M. Bifurcata and M. diana* were sequenced at Bio-Transduction Lab Co.Ltd. (Wuhan, China) by Sanger sequencing. PCR primers were designed according to conserved region sequences and used to amplify the mitochondrial DNA sequence in PCR reactions (Table 3, 4). The PCR reaction was performed using the LA Taq polymerase. The thermal cycling conditions comprised an initial denaturation step at 94°C for 2 min, then 35 cycles of denaturation at 94 °C for 30 s, 30 s for annealing at 55°C, and elongation at 72 °C for 1 min/kb, followed by the final extension at 72 °C for 10 min. The PCR products were purified and sequenced using an ABI 3730 automatic sequencer. After quality-proofing of the obtained DNA fragments, and BLASTed were used to confirm that the amplification is the actual target sequence (*Meng et al., 2013*; *Yu et al., 2017*). The complete mitogenome sequence was assembled manually through DNAStar v7.1 (*Burland, 2000*).

Genome Annotation and Analyses

First of all, raw mitogenomic sequences were entered into MITOS web servers

(http://mitos.bioinf.uni-leipzig.de/index.py, accessed on 15 Jun 2021) in an effort to fix the rough boundaries of genes. Accurate locations of protein-coding genes (PCGs) were determined by seeking ORFs (employing genetic code 5, the invertebrate mitochondrion). All tRNAs were characteried by using tRNAscan SE v. 1.21 and ARWEN (*Lowe et al., 1997; Laslett et al., 2008*). The precise boundaries of *rrnL* and *rrnS* were defined by homologous comparison. Genomes manually annotated were parsed and extracted by means of PhyloSuite, and GenBank (NCBI) submission files and organization tables for mitogenomes were also created through the same software (*Zhang et al., 2020*).

The mitogenomic circular map was generated by OrganellarGenomeDRAW (OGDRAW) version 1.3.1 (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html, accessed on 3 March 2023 (*Greiner et al., 2019*). Intergenic spacers and overlapping regions between genes were performed manually. The nucleotide base composition, codon usage, as well as values of A + T content were calculated with MEGA 11.0 (*Tamura et al., 2021*). The bias of nucleotide composition was computed according to AT skew = [A – T] / [A + T] and GC skew = [G – C] / [G + C] (*Perna & Kocher, 1995*). Additionally, the nucleotide diversity (Pi) and nonsynonymous (Ka)/synonymous (Ks) mutation rate ratios were operated by DNAsp 6.0 (*Julio et al., 2017*).

Phylogenetic analysis

A molecular phylogenetic analysis was constructed on the basis of mitogenomes of 28 species and two species regarded as outgroups (Table 2). All complete mitochondrial sequences were selected to accomplish phylogenetic analyses. The Gblocks version 0.91b was adopted to clean out the gaps and fuzzy-alignment sites, and all alignments were verified and revised in MEGA 11.0 prior to phylogenetic analysis (*Tamura et al., 2021*). The phylogenetic trees were constructed by

introducing two methods both the maximum likelihood (ML) method and the Bayesian Inference (BI) method (*Nguyen et al., 2015*; *Zhou et al., 2011*). The ML analysis was performed with IQ-TREE under a ML+rapid bootstrap (BS) algorithm with 10,000 replicates used to calculate bootstrap scores for each node (BP). The BI analysis was carried out using MrBayes 3.2.7 elected GTR+G+I as the optimal model, running 10 million generations, sampling every 1000 trees, 25% of samples were abandoned as burn-in.

Results and discussion

Taxonomy based on morphology

***Arboridia* (*Arboridia*) *rongchangensis* Zhang & Song, sp. nov. (Figures 1-2)**

Description. Dorsum dark brownish (Figs. 1a, 1c). Color pattern brown. Head narrower than pronotum (Fig. 1a, 1c). Crown fore margin weakly produced medially. Vertex with a pair of dark preapical spots. Face yellowish white, with anteclypeus narrow and pale, and frontoclypeus dark (Fig. 1b, 1d). Pronotum wide, scutellum with dark lateral triangles (Figs. 1a, 1c). Forewings without spots or markings.

Male genitalia. Pygofer dorsal appendage simple, without branch, hook-like apically (Figs. 2e, 2f). Subgenital plate with 2 macrosetae on lateral surface, and row of peg-like setae from subbase to apex, and several microsetae scattered on apical portion (Figs. 2d). Style long and slender, with 2 points apically; preapical lobe obtuse and distinct (Fig. 2a). Aedeagus with a large lamellate process arising from base of aedeagal shaft ventrally; aedeagal shaft broad and flat, slightly bifurcated at apex; gonopore subapical on ventral surface; preatrium little longer than shaft (Figs. 2b, 2c). Connective V-shaped, with arms long (Fig. 2g).

Male abdominal apodemes small, not exceeding 3rd sternite (Fig. 2h).

Commented [MW4]: Make colour and structure separate paragraphs

Deleted: obvious

Deleted: one

Deleted: two

Deleted: arms

Measurement. Male length 3.0~3.1 mm, female length 3.1~3.2 (including wings).

Specimens examined. Holotype: ♂, CHINA, Chongqing, Rongchang, 14 Ⅷ 2021, coll. Guimei Luo. Paratypes: 11 ♂ ♂, 32 ♀ ♀, same data as holotype.

Remarks. This species is similar to *Arboridia reniformis* Song & Li (*2013*), but differs in having the aedeagal shaft distinctly bifurcate apically.

Etymology. The new species is named after its type locality: "Rongchang" county, Chongqing, China.

***Thaia* (*Thaia*) *jiulongensis* Zhang & Song, sp. nov. (Figures 3-4)**

Description. Vertex yellow, light brownish in middle apically (Figs. 3a, 3c). Face yellowish brown (Figs. 3b, 3d). Pronotum orange brown, with pair of large triangular impressions, posterior and anterior part lighter, yellowish (Figs. 3a, 3c). Scutellum with anterior margin yellow and posterior part milky yellow; lateral triangles brownish black (Figs. 3a, 3c).

Male abdominal apodeme small, not surpassing 3rd sternite (Fig. 4i).

Male genitalia. Pygofer lobe with scattered fine microsetae on dorsal surface, with dorso-caudal margin angulated (Fig. 4f). Anal tube with well-developed basal appendages, extending ventro-caudally (Fig. 4g). Subgenital plate broadened at subbase, provided with 3 macrosetae on lateral surface at midlength, numerous peg-like small setae along dorsal margin from near midlength part to apex; several small setae scattered apically (Figs. 4e, 4f). Style slender apically, preapical lobe well developed (Fig. 4a). Aedeagus expanded at base in ventral view, with pair of long basal process arising from preatrium ventrally, which slim and curved, tapering towards apex; gonopore apical on ventral surface (Figs. 4b, 4c, 4d). Connective V-shaped, without central lobe, lateral arms long and slim (Fig. 4h).

Deleted:

Deleted: us

Deleted: with obvious

Deleted: bifurcation apically

Commented [MW5]: Add external characters mentioned in the previous species

Commented [MW6]: What does this mean?

Deleted: ,

Deleted: with anteclypeus ovoid

Deleted: centrally

Deleted: middle

Commented [MW7]: Does this mean the figures are the wrong way round? Also where is atrium?

Commented [MW8]: ditto

Specimen examined. Holotype: ♂, CHINA, Chongqing, Jiulongpo District, Zhongliang Yunling Forest Park, 14 Ⅷ, 2021. coll. Weiwen Tan. Paratypes: 5♂♂, 15♀♀, same data as holotype.

Measurement. Body length ♂ 3.0~3.2 mm; ♀ 3.0~3.2 mm (including wings).

Remarks. This species is similar to *Thaia* (*Thaia*) *barbata* Dworakowska (*1979*), but can be distinguished from the latter species by from the shape of the adedeagus with a small subapical protrusion and the anal tube appendages which are particularly wide.

Deleted:

Deleted: ,

Deleted: , connective. Its adedeagal shaft has a small protrusion near the top. The anal tube appendages look like a glove,

Deleted: is

Deleted: compared to *T.* (*T.*) *barbata* Dworakowska (*1979*). Connective arms are relatively slender.

Deleted: M

Etymology. The new species is named after its type locality, Jiulong, Chongqing.

Taxonomy based on molecular data

Organization and composition of the genome

Just as other previously reported species in Typhlocybinae, the genomic structure and nucleotide composition of a couple of mitogenomes sequenced in the present study are quite similar (*Chen et al., 2021a; Chen et al., 2021b*). The complete mitogenomes of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* are 15,596, 15676, 16,183 and 16,589 bp, separately. Both species comprise 13 PCGs, 22 tRNA genes, two rRNA genes, and a control region (CR) (Fig. 5). Two strands, the majority strand (H-strand) and the minority strand (L-strand), exist in the mitochondrial genome. The H-strand consists of 23 genes (9 PCGs, 14 tRNAs) and CR, and meanwhile, the L-strand encompasses 14 genes (4 PCGs, 8 tRNAs, and 2 rRNAs).

There are 50 bp, 66 bp, and 70 bp intergenic spaces presented in total length of all the intergenic space ranging from 1 to 10 bp, 1 to 13 bp, 1 to 9 bp in *A.* (*A.*) *rongchangensis* sp. nov., *M. bifurcata*, *M. diana*. However, 52 bp intergenic space existed in 11 regions from 1 to 15 bp in *T.* (*T.*) *jiulongensis* sp. nov. It can be observed that ten genes overlapped by 28 bp in *A.* (*A.*) *rongchangensis* sp. nov., eleven genes overlapped by 31 bp in *T.* (*T.*) *jiulongensis* sp. nov., ten genes overlapped by a grand total of 32 bp in *M. bifurcata*, nine genes overlapped by 40 bp *M. diana* (Table 5). Such similar gene structure is common among leafhoppers (*Chen et al., 2021a; Yuan et al., 202*1; *Wang et al., 2021*).

The heavy AT nucleotide bias appears in the mitochondrial genomes in *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana*, the A+T contents are 80.7%, 78.0%, 78.4% and 78.5%, respectively (Table 5). These four species showed similar compositions involving positive AT skews and negative GC skews. These results manifested that the content of base A exceeds base T. Despite the AT skews being positive, a few genes have a slight difference in absolute values. In addition, negative GC skew manifested that the content of base G is below that of base C, positive values are just the opposite. On balance, the essential organization of four species tends to be base A and C.

Protein-coding genes and codon usage

As with most other Typhlocybinae, the overall length of 13 PCGs of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* are 10,946, 10,968, 10,966 bp and 10,966 bp, 70.3%, 69.8%, 65.20% and 66.1% of the total genome of each species, respectively. In addition, *nad2* and *cox3* in four species have the same start codons and stop codons. The longest PCG is *nad5* (1,675 bp) in *A.* (*A.*) *rongchangensis* sp. nov., the shortest is *atp8* (144 bp) in *M. bifurcata* and *M. diana*. Only four genes (*nad5*, *nad4*, *nad4L* and *nad1*) are presented on the J-strand, and the remaining nine genes are presented on the H-strand.

The relative synonymous codon usage (RSCU) values of the 13 PCGs are generalized in Fig. 6. The codon usage analyses of *A.* (*A.*) rongchangensis sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* revealed that codon UUA-Leu2 (214, 194, 180, 241), AUU-Ile (297, 249, 274, 210), AUA-Met (245, 202, 223, 180) AAU-Asn (273, 239, 236, 256), and AAA-Lys (290, 280, 227, 238) are the most frequently used (Fig. 9). The highest RSCU value of each species occur in UUA-Leu2. The results showed that UUA is the most preferred codon. In addition, it can be seen from the RSCU values of the PCGs that AT is used more frequently than GC.

Transfer RNA and ribosomal RNA genes

The 22 tRNAs were deconcentrated between two regions, the rRNAs and the protein-coding region. The total tRNA lengths of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* are 1,434, 1,436, 1,441 and 1,455 bp, respectively, which range from 61 to 70 bp in *A.* (*A.*) *rongchangensis* sp. nov., 61 to 71 in *T.* (*T.*) *jiulongensis* sp. nov., 61 to 71 bp in *M. bifurcata* and 62 to 71 bp in *M. diana* (Table 5). The sequences of most tRNA genes demonstrated the exemplary clover-leaf secondary structure, including four structural domains and a short flexible

loop: the acceptor stem, the dihydrouridine stem and loop (DHU), the anticodon stem (DHU) and loop, the thymidine stem and loop (TψC), and the variable (V) loop (Fig. S1), as observed in many other leafhoppers mitogenomes. However, the dihydrouridine (DHU) arm of *trnS1* shapes an uncomplicated loop. Additionally, non-Waston-Crick base pairs were harbored the stems of the secondary structures, 21, 27, 21 and 18 weak G-U (or U-G) base pairs are revealed in the tRNAs of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* (Fig. S1). The location of mismatched base pairs in the acceptor arm, DHU arm, TψC arm and anticodon arm of tRNA from four species were shown in Table 6. These mismatches could be rectified by way of editing process, and the transport function is not influenced (*Yuan et al., 2021*).

Like other insects (*Wang et al., 2018*), the two rRNAs (*rrnL* and *rrnS*) are placed in *trnL* and control regions, separated by *trnV* (Fig. 5). The lengths of rrnL genes of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* mitogenomes are 1,916 bp, 1,927 bp, 1,186 bp and 1,186 bp, the A+T content is 83.0%, 81.6%, 83.0%, 82.8% with values in different directions, including positive AT skew (0.198, 0.221, 0.186, 0.198) and negative GC skew (-0.302, -0.276, -0.294, -0.256), respectively.

Control region

The control region, also known as the A+T region, acts a crucial part in the size variation of mitogenomes. The largest non-coding regions of the two species, putative control regions, were placed between *rrnS* and *trnI*. The control region in length of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana* are 1,276 bp, 1,345 bp, 2,472 bp and 2,233 bp, the AT contents are 99.0%, 97.9%, 89.9% and 92.0%, respectively.

Nucleotide diversity and evolutionary rate analysis

Highly variable nucleotide diversity (Pi values) from 13 PCG sequences of the 28 mitogenomes is shown in Fig. 8, with values ranging from 0.195 (cox1) to 0.418 (*atp8*). The genes *nad4* (Pi = 0.418), *nad2* (Pi = 0.358), *atp8* (Pi = 0.349), *nad1* (Pi = 0.320), *nad6* (Pi = 0.274) and *nad3* (Pi = 0.251) have comparatively high nucleotide diversity. However, the genes *nad5* (Pi = 0.272), *cox2* (Pi = 0.243), *cox3* (Pi = 0.235), *nad4L* (Pi = 0.239), *cytb* (Pi = 0.225) and *cox1* (Pi = 0.195) share comparatively low values of nucleotide diversity.

The evolutionary rate was measured through average non-synonymous (Ka)/synonymous (Ks) substitution rates by the instrumentality of DNAsp 6.0. The pairwise Ka/Ks analysis shows that the

Ka/Ks ratios of *nad3* (2.011), *nad4L* (1.843), *cox1* (1.182) are greater than 1, indicating that these genes are under positive selection. Whereas the ratios of remaining genes ranged from *cytb* (0.390) to *atp8* (0.824) (Fig 7), demonstrating that these genes are confined in purifying selection. Nucleotide diversity and evolutionary rate analysis are usually used for identifying the regions with large nucleotide divergence, and play an important role in designing species-specific markers (*Yu et al., 2015*). These evolving more quickly genes, *cox1*, *nad3* and *nad4L*, may be speculated as potential DNA markers to delimit the Typhlocybinae species.

Phylogenetic analysis

In this study, complete mitochondrial genomes from 28 Typhlocybine species were collected as a dataset to establish phylogenetic trees by BI and ML methods, *Bothrogonia ferruginea* and *Iassus dorsalis* were regarded as outgroups. The GenBank accession numbers of all selected species used in this study were listed in Table 2. The phylogenetic topologies constructed by the two methods were completely consistent (Fig. 8). The monophyly of each tribe was generally well supported in the subfamily Typhlocybinae, which is consistent with the findings of some previous molecular phylogenetic studies (*Chen et al., 2021a; Chen et al., 2021b*). Twelve species of Typhlocybini, twelve species of Erythroneurini, and four species of Empoascini are clustered together, respectively, and all phylogenetic relationships demonstrated higher nodal support in both ML and BI analyses. All species from Typhlocybinae (Inner group) are clustered together, all *Mitjaevia* species are gathered together. Our results further confirmed that the genus *Arboridia* has a closer relationship with *Mitjaevia*. Among them, *M. bifurcata*, *M. protuberanta* and *M. diana* are gathered into one clade, while *M. bifurcata* and *M. protuberanta* are sister groups of each other in ML tree and BI tree. The previous primary diagnosis of Typhlocybinae was made by morphological features, meanwhile, our phylogenetic tree based on molecular data is in agreement with morphological taxonomy. Because the external appearance of *Mitjaevia* species is very similar, the only difference lies in male genitalia including the pygofer, subgenital plate and aedeagus, so, molecular technologies have become particularly important as a supplement to identification of *Mitjaevia* species. This study indicated that mitochondrial genome sequences are the most popularly adopted genomic markers in leafhoppers and becoming increasingly important toward studies in the insect molecular field, involving molecular evolution, phylogeny and phylogeography.

Discussion

The traditional classification of leafhoppers mainly relies on the anatomy of their appearance and male genitalia (*Song & Li, 2013; Ramaiah et al., 2023; Xu & Zhang, 2023*). However, due to the large number of leafhoppers and the small size of the Erythroneurini leafhoppers, generally about 2~4 mm (*Dietrich & Dmitriev, 2006*), they are difficult to identify. In recent years, the development of molecular technology has been applied to the classification of insects *(Singh et al., 2017; Lu et al., 2018; Matsuia et al., 2022)* and to support the results and correct the attribution of traditional classification. Our findings here on the complete mitochondrial genome supports the classification of two new species.

In addition, we also sequenced and analyzed the mitochondria genomes of two *Mitjaevia* genera, this result enriches the mitochondrial database information of Cicadellidae family and is consistent with the results of previous articles published by our research group *(Chen et al., 2021b)*. In the Typhlocybinae, each genus is divided into a separate branch, this result is consistent with previous results (*Lin et al., 2021*), and all *Mitjaevia* are clustered in one branch. The phylogenetic relationship between the two new species is closer to that of *Mitjaevia*. This may be due to the limited mitochondrial data currently sequenced in Erythroneurini, which requires more and more extensive mitochondrial data to support and elucidate the phylogenetic relationship of the new species. The mitochondrial data can not only confirm the correctness of traditional classification, but also establish a large database, and provide simpler, faster, and more efficient results for subsequent species classification.

Conclusions

Two new leafhopper species discovered in Chongqing, *A.* (*A.*) *rongchangensis* sp. nov. and *T.* (*T.*) *jiulongensis* sp. nov. are described and illustrated. The mitochondrial genomes of these species together with *M. bifurcata* and *M. diana* are assembled and annotated in this work. The study shows that their mitogenomes are conserved in structure, with length of 15,596 bp, 15,676 bp, 16,813 bp and 16,589 bp, including 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. The PCGs begin with ATA/ATG/ATT/TTG, and cease with TAA/TAG/T. All tRNAs are folded into a typical clover-leaf secondary structure, except a few tRNAs with a reduced arm, offering a simple loop or constituted unpaired bases. Based on the morphology of leafhoppers, the phylogenetic tree also further confirms the relationship between the four species at the molecular level, the result also indicates consistency in molecular and traditional classification.

Deleted: Based on morphological characters, *A.* (*A.*) *rongchangensis* Zhang & Song, sp. nov. can be differentiated from *A. reniformis* in having the aedeagus with obvious bifurcation apically (*Song & Li, 2013*). *T.* (*T.*) *jiulongensis* Zhang & Song, sp. nov. is similar to *T.* (*T.*) *barbata* Dworakowska (1979), but can be distinguished from the shape of the adedeagus, anal tube appendages, connective (*Dworakowska, 1979*).

Deleted: it is

Deleted: the Erythroneurini leafhoppers, which is improper to depend on the traditional morphological classification

Deleted: . It can

Deleted: of traditional classification

Deleted: T

Deleted: ,

Deleted: *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov.

Deleted: ,

Deleted: of *A.* (*A.*) *rongchangensis* sp. nov., *T.* (*T.*) *jiulongensis* sp. nov., *M. bifurcata* and *M. diana*

Funding: This research was funded by the World Top Discipline Program of Guizhou Province: Karst Ecoenvironment Sciences (No. 125 2019 Qianjiao Keyan Fa), the Innovation Group Project of Education Department of Guizhou Province ([2021]013), the National Natural Science Foundation of China (32260120), the Natural Science Foundation of Guizhou Province (Qiankehejichu-ZK [2023] General 257) and the Training Program for High-level Innovative Talents of Guizhou Province ([2023]0032).

Data Availability Statement: The data supporting the findings of this study are openly available in National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov), accession numbers were OK448488 (*Mitjaevia bifurcata*), OK448489 (*Mitjaevia diana*), OQ404948 (*Arboridia* (*Arboridia*) *rongchangensis* sp. nov) and OQ630475.1 (*Thaia* (*Thaia*) *jiulongensis* sp. nov.). SRA: PRJNA1007413 (*Arboridia* (*Arboridia*) *rongchangensis* sp. nov), PRJNA1007448 (*Thaia* (*Thaia*) *jiulongensis* sp. nov.).

Conflicts of Interest: The authors declare no conflict of interest.

Reference

Almeida CE, Marct PL, Gumiel M, Takiya DM, Costa J. 2009. Phylogenetic and phenotypic relationships among triatoma carcavalloi (hemiptera: reduviidae: triatominae) and related species collected in domiciles in rio grande do sul state, brazil. *Journal of Vector Ecology* **34(2):** 164–173 DOI 10.1111/j.1948-7134.2009.00023.x.

Bellota E. 2011. Feeding and oviposition performances of corn leafhopper, along maize's evolutionary and domestication gradients. *Journal of Chromatography B Biomedical Sciences & Applications* **571(s 1-2):** 291–297 DOI 10.1016/0378-4347(91)80458-O.

Bosco D, Galetto L, Leoncini P, Saracco P, Raccah B, Marzachì C. 2007. Interrelationships between "Candidatus Phytoplasma asteris" and its leafhopper vectors (Homoptera: Cicadellidae). *Journal of Economic Entomology* **(5):**1504–1515 DOI 10.1603/0022-0493-100.5.1504.

Burland TG. 2000. DNASTAR's Laser Gene sequence analysis software. *Methods in Molecular Biology* **132,** 71– 91. DOI 10.1385/1-59259-192-2:71.

Chen L, Feng Y, Chen HM, Wang LX, Feng HL, Yang X, Mughal MN, Fang R. 2016. Complete mitochondrial genome analysis of Clinostomum complanatum and its comparison with selected digeneans. *Parasitology Research* **115(8):** 3249–3256 DOI 10.1007/s00436-016-5102-6.

Chen XS, Li ZZ. 2004. Cladistics analysis of the phylogenetic relationships among the genera of *nirvaninae* (homoptera:cicadellidae) from China. *Journal of Mountain Agriculture and Biology* **23,** 113–120.

Chen XX, Yuan Z, Li C, Dietrich CH, Song Y. 2021a. Structural features and phylogenetic implications of Cicadellidae subfamily and two new mitogenomes leafhoppers. *Plos One* **16,** e0251207 DOI 10.1371/journal.pone.0251207.

Chen XX, Li C, Song YH. 2021b. The complete mitochondrial genomes of two erythroneurine leafhoppers (Hemiptera, Cicadellidae, Typhlocybinae, Erythroneurini) with assessment of the

phylogenetic status and relationships of tribes of Typhlocybinae. *ZooKeys* **1037:** 137–159. DOI 10.3897/zookeys.1037.63671.

Chen XX, Yuan ZW, Li C, Song YH. 2020. Complete mitochondrial genome sequence of *Empoascanara dwalata* (Hemiptera: Cicadellidae: Typhlocybinae). *Mitochondrial DNA Part B-resources* **5(3):** 2260–2261 DOI 10.1080/23802359.2020.1772141.

Dietrich CH, Dmitriev DA. 2006. Review of the New World genera of the leafhopper tribe Erythroneurini (Hemiptera: Cicadellidae: Typhlocybinae). *Champaign ILL Illinois Natural History Survey* **37,** 119–190.

Distant WL. 1918. Rhynchota. VII. Homoptera: appendix. Heteroptera: addenda. *The fauna of British India, including Ceylon and Burma. London: Taylor & Francis* **8,** 1–210.

Dmitriev DA. 2003. onward: 3I Interactive Keys and Taxonomic Databases. Available online: http://dmitriev.speciesfi le.org/index. asp (accessed on 25 April 2023).

Dworakowska I. 1970. On the genera Asianidia Zachv. and Singapora Mahm. with the description of two new genera (Auchenorrhyncha, Cicadellidae, Typhlocybinae). *Bulletin de l'Academie Polonaise des Sciences* **(18):** 759–765.

Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acid Research* **47(W1):** W59-W64 DOI 10.1101/545509.

Jiang J, Chen XX, Li C, Song Y. 2021. Mitogenome and phylogenetic analysis of typhlocybine leafhoppers (hemiptera: cicadellidae). *Scientific Reports* **11(1):** 10053 DOI 10.1038/s41598-021-89525-5.

Laslett D, Canbck AB. 2008. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics* **24,** 172–175. DOI 10.1093/bioinformatics/btm573.

Lin SH, Huang M, Zhang YL. 2021. Structural features and phylogenetic implications of 11 new mitogenomes of Typhlocybinae (Hemiptera: Cicadellidae). *Insects* **12(8):** 678 DOI 10.3390/insects12080678.

Longo SJ, Faircloth BC, Meyer A, Westneat MW, Alfro ME, Wainwright PC. 2017. Phylogenomic analysis of a rapid radiation of misfit fishes (Syngnathiformes) using ultraconserved elements. *Molecular Phylogenetics Evolution* **113,** 1–57 DOI 10.1016/j.ympev.2017.05.002.

Lowe TM, Eddy SR. 1997. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* **25,** 955–964 DOI 10.1093/nar/25.5.0955.

Lu YJ, Zhao YR, Wang ZY, Miao SY, Liu FJ, Emery RN. 2018. DNA barcoding, species-specific PCR for the identification of three stored-product pest species of genus Palorus (Coleoptera: Tenebrionidae). *Journal Stored Products Research* **78:** 32–38. DOI 10.1016/j.jspr.2018.06.001.

Luo GM, Song QF, Song YH. 2021. Two new species of the leafhopper genus *Mitjaevia* Dworakowska from China (Hemiptera, Cicadellidae, Typhlocybinae). *Biodiversity Data Journal* **9,** e72420 DOI 10.3897/BDJ.9.e72420.

Luo X, Chen Y, Chen C, Pu D, Tang X, Zhang H, Lu D, Mao J. 2019. Characterization of the complete mitochondrial genome of *Empoasca sp*. (Cicadelliae: Hemiptera). *Mitochondrial DNA Part B-Resources* **4(1):** 1477–1478 DOI 10.1080/23802359.2019.1579066.

Luo ZH, Tang SH, Jiang ZG, Chen J, Fang HX, Li CW. 2016. Conservation of Terrestrial Vertebrates in a Global Hotspot of Karst Area in Southwestern China. *Scientific Reports* **6,** 25717. DOI: 10.1038/srep25717.

Matsuia Y, Mallyb R, Kohama S, Aokid I, Azumaa M, Nakaa H. 2022. Molecular phylogenetics and tribal classification of Japanese Pyraustinae and Spilomelinae (Lepidoptera: Crambidae). *Insect Systematics & Evolution* **54(1):** 77–106 DOI10.1163/1876312X-bja10037.

Meng ZH, Yang MF, Zhou YF, Lv ZY, Ni JQ. Molecular phylogenetic analysis of some species of Cicadellinae based on mitochondrial Cytb gene. *Guizhou Agricutural Science* **41(7):** 1–5.

Morris MG. 1971. Differences between the invertebrate faunas of grazed and ungrazed chalk grassland, IV. Abundance and diversity of Homoptera–Auchenorrhyncha. *Journal of Applied Entomology* 8, 37–52.

Nguyen LT, Schmidt HA, Von HA, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology & Evolution* **(1):** 268–274 DOI 10.1093/molbev/msu300.

Peng Y, Leung HCM, Yiu SM, Chin FYL. 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* **28,** 1420–1428. DOI 10.1093/bioinformatics/bts174.

Perna NT, Kocher TD. 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Journal of Molecular Evolution* **41,** 353–358.

Ramaiah M, Meshram NM, Dey D. 2023. A new species of bamboo-feeding leafhopper genus Mukariella Viraktamath and Webb (Hemiptera: Cicadellidae) along with its morphological variant from India. *Zootaxa* **5239(2):** 289–295 DOI 10.11646/zootaxa.5239.2.7.

Roddee J, Kobori Y, Hanboonsong Y. 2018. Multiplication and distmribution of sugarcane white leaf phytoplasma transmitted by the leafhopper, *Matsumuratettix hiroglyphicus* (Matsumura) (Hemiptera: Cicadellidae), in infected sugarcane. *Sugar Tech* **20,** 445–453 (2018). DOI 10.1007/s12355-017-0559-x.

Julio R, Albert FM, Juan CSD, Sara GR, Pablo L, Sebastián ERO, Alejandro SG. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology & Evolution* **34,** 3299–3302 DOI 10.1093/molbev/msx248.

Singh D, Kabiraj D, Sharma P, Chetia H, Mosahari PV, Neog K, Bora U. 2017. The mitochondrial genome of Muga silkworm (*Antheraea assamensis*) and its comparative analysis with other lepidopteran insects. *Plos one* **12(11):** e0188077 DOI 10.1371/journal.pone.0188077.

Song N, Zhang H, Zhao, T. 2019. Insights into the phylogeny of Hemiptera from increased mitogenomic taxon sampling. *Mol. Phylogenet. Evol.* **2019,** *137*, 236–249.

Song YH, Li ZZ, Dai RH. 2016. A remarkable new genus and species of Erythroneurini (Hemiptera: Cicadellidae: Typhlocybinae) from China. *Florida Entomologist* **99(3):** 371–375 DOI 10.1653/024.099.0305.

Song YH, Li C. 2017. A new erythroneurine leafhopper genus and species from Thailand (Hemiptera: Cicadellidae: Typhlocybinae). *Zootaxa* **4319(2):** 379–382 DOI 10.11646/Zootaxa.4319.2.9.

Song YH, Li ZZ. 2013. Some new species and new record of the genus *Arboridia* Zachvatkin (Hemiptera: Cicadellidae: Typhlocybinae) from six provinces of China. *Zootaxa* **3613(3):** 229–244 DOI 10.11646/zootaxa.3613.3.2.

Song YH, Li ZZ, Xiong KN. 2011. Two new species of the genus *Mitjaevia* Dworakowska from China (Hemiptera: Cicadellidae: Typhlocybinae). Zootaxa **2805:** 26–30.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11.0. *Molecular Biology & Evolution* **38(7):** 3022–3027 DOI 10.1093/molbev/msab120.

Tan C, Chen XX, Li C, Song YH. 2020. The complete mitochondrial genome of *Empoascanara sipra* (Hemiptera: Cicadellidae: Typhlocybinae) with phylogenetic consideration. *Mitochondrial DNA Part B-resource* **5(1):** 260–261 DOI 10.1080/23802359.2019.1698990.

Wang B, Szwedo J, Zhang H, Fang Y. 2010. New Froghoppers from the Jurassic of China and Their Phylogenetic Significance (Insecta: Hemiptera: Cercopoidea). *Earth Science Frontiers* **17(Special Issue),** 224–225.

Wang JJ, Wu YF, Yang MF, Dai RH. 2020a. The phylogenetic implications of the mitochondrial genomes of *macropsis notata* and *oncopsis nigrofasciata*. *Frontiers in Genetics* **11,** 443. DOI 10.3389/fgene.2020.00443.

Wang JJ, Wu YF, Dai RH, Yang MF. 2020b. Comparative mitogenomes of six species in the subfamily Iassinae (Hemiptera: Cicadellidae) and phylogenetic analysis. *International Journal of Biological Macromolecules* **149,** 1294–1303 DOI 10.1016/j.ijbiomac.2020.01.270.

Wang XY, Wang JJ, Dai RH. 2021. Structural features of the mitogenome of the leafhopper genus *Cladolidia* (Hemiptera: Cicadellidae: Coelidiinae) and phylogenetic implications in Cicadellidae. *Ecology & Evolution* **11(18):** 12554–12566 DOI 10.1002/ece3.8001.

Wang ZF, Wang Z, Shi X, Wu Q, Tao Y, Guo H, Ji C, Bai Y. 2018. Complete mitochondrial genome of Parasesarma affine (Brachyura: Sesarmidae): Gene rearrangements in Sesarmidae and phylogenetic analysis of the Brachyura. *International Journal of Biological Macromolecules* **118,** 31–40 DOI 10.1016/j.ijbiomac.2018.06.056.

Womack CL, Schuster MF. 1986. Potato Leafhopper (Homoptera: Cicadellidae) Feeding on Seedling Cotton. *Journal of Economic Entomolog* **5,** 1259–1263 DOI 10.1093/jee/79.5.1259.

Xiong KN, Xiao SZ, Liu ZQ, Chen PD. 2008. A Comparative Analysis of the Value of the World Natural Heritage of the Karst in South China. Engineering Sciences. **10,** 17-28.

Xu DL, Zhang YL. 2023. Taxonomy of the leafhopper genus *Drabescus* Stal (Hemiptera: Cicadellidae: Deltocephalinae) with description of two new species from China. *Zootaxa* 2023, **5264**, 393–404. DOI 10.11646/zootaxa.5264.3.7.

Yan B, Dietrich CH, Yu XF, Jiao M, Dai RH, Yang MF. 2022. Mitogenomic phylogeny of Typhlocybinae (Hemiptera: Cicadellidae) reveals homoplasy in tribal diagnostic morphological traits. *Ecology & Evolution* **12(6):** e8982 DOI 10.1002/ece3.8982.

Yang X, Yuan ZW, Li C, Song YH. 2020. Complete mitochondrial genome of *Eupteryx* (*Stacla*) minusula (Hemiptera: Cicadellidae: Typhlocybinae) from China. *Mitochondrial DNA Part B-Resources 5*(3): 2375–2376. DOI 10.1080/23802359.2020.1775146.

Yao GA, Zhang YL, Dietrich CH, Duan YN. 2021. Phylogenetic analyses and species delimitation of *nephotettix matsumura* (Hemiptera: Cicadellidae: Deltocephalinae: Chiasmini) in China based on molecular data. *Zoologischer Anzeiger* **293,** 202–214 DOI 10.1016/j.jcz.2021.06.011.

Young DA. 1952. A reclassification of Western Hemisphere Typhlocybinae (Homoptera, Cicadellidae). Univ. Kans. Sci. Bull. **35,** 3–217 DOI 10.5962/bhl.part.4327.

Yu GC, Tang QZ, Long KR, Che TD, Li MZ, Shuai SR. 2015. Effectiveness of microsatellite and single nucleotide polymorphism markers for parentage analysis in European domestic pigs. *Genetics and Molecular Research* 14(1): 1362-1370 DOI 10.4238/2015.February.13.15.

Yu PF, Wu GH, Han BY. 2017. Sequencing and analysis of the mitochondrial genome of *Kolla paulula* (Walker) (Hemiptera: Cicadellidae). *Journal of Anhui Agricultural University* **44(5):** 874–881 DOI 10.13610/j.cnki.1672-352x 20171017.032.

Yu PF, Li Q, Wang MX, Pan C, Cui L, Han BY. 2019. Analysis of complete mitochondrial genome and phylogenetic relationship of bothrogonia ferruginea. *Journal of Agricultural Biotechnology* 27(07): 1246–1258.

Yuan LL, Ge X, Xie G, Liu H, Yang Y. 2021. First complete mitochondrial genome of Melyridae (Coleoptera, Cleroidea): genome description and phylogenetic implications. *Insects* 12(2): 87 DOI 10.3390/insects12020087.

Yuan XW, Xiong KN, Li C, Song YH. 2020. The complete mitochondrial genome of *Limassolla lingchuanensis* (Hemiptera: Cicadellidae: Typhlocybinae). *Mitochondrial DNA Part B-Resources* **5(1):** 229–230. DOI 10.1080/23802359.2019.1698354.

Yuan XW, Li C, Song YH. 2020. Characterization of the complete mitochondrial genome of *Mitjaevia protuberanta* (Hemiptera: Cicadellidae: Typhlocybinae). *Mitochondrial DNA Part B-resource* **5(1):** 601–602 DOI 10.1080/23802359.2019.1710601.

Yuan ZW, Xiong KN, Zhang N, Li C, Song YH. 2021. Characterization of the morphology and complete mitochondrial genomes of *Eupteryx minusula* and *Eupteryx gracilirama* (Hemiptera: Cicadellidae: Typhlocybinae) from Karst area, Southwest China. *PeerJ* **9,** e12501 DOI 10.7717/peerj.12501.

Zhang D, Gao F, Jakovli I, Zou H, Wang GT. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* **20,** 348–355 DOI 10.1111/1755-0998.13096.

Zhang N, Song QF, Song YH. 2021. A new leafhopper genus of Erythroneurini (Hemiptera, Cicadellidae, Typhlocybinae) from karst area in southwestern China. *Revista Brasileira de Entomologia* **65(4):** e20210041 DOI 10.1590/1806-9665-RBENT-2021-0041.

Zhou JF, Liu XG, Stones DS, Xie Q, Wang G. 2011. MrBayes on a graphics processing unit. *Bioinformatics (Oxford, England)* **27:** 1255–1261 DOI 10.1093/bioinformatics/btr140.

Zhou N, Wang M, Cui L, Chen X, Han B. 2016. Complete mitochondrial genome of *Empoasca vitis* (Hemiptera: Cicadellidae). *Mitochondrial DNA Part A* **27(2):** 1052–1053 DOI 10.3109/19401736.2014.928863.

Zhou X, Dietrich CH, Huang M. 2020. Characterization of the complete mitochondrial genomes of two species with preliminary investigation on phylogenetic status of Zyginellini (Hemiptera: Cicadellidae: Typhlocybinae) *Insects* 11(10): 684 DOI 10.3390/insects11100684.

Zhou X, Li Y, Liu S, Yang Q, Su X, Zhou L, Tang M, Fu R, Li J, Huang Q. 2013. Ultradeep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. *GigaScience 2*(1): 1–12. DOI 10.1186/2047-217X-2-4.

Zhu H. 2007. The karst ecosystem of southern China and its biodiversity. *Tropical forestry* **35(S1):** 44–47.

Zhu X, Liu H, Wu L, Liang B, He W. 2020. Impact of bedrock geochemistry on vegetation productivity depends on climate dryness in the guizhou karst of china. *Progress in Physical Geography* **45(12)**: 030913332093608 DOI 10.1177/0309133320936085.