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Phylogenetic and mitochondrial genome analysis of a putative new cave cricket species (Rhaphidophoridae) from a Thai subterranean habitat

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Abstract. *Inchoedchay K, Homchan S, Gupta YM. 2025. Phylogenetic and mitochondrial genome analysis of a putative new cave cricket species (Rhaphidophoridae) from a Thai subterranean habitat. Biodiversitas 26: 3855-3862.* Cave crickets (family Rhaphidophoridae) are a unique group of orthopteran insects characterized by long legs, large hind legs, elongated antennae, a humped back body, and are wingless, adaptations that enable them to thrive in subterranean environments such as caves. Despite their ecological importance and unique adaptations, their mitochondrial genomic architecture remains poorly characterized. This study investigates the mitochondrial genome of a cave cricket from the family Rhaphidophoridae, commonly referred to as cave crickets. Specifically, this research presents the first complete mitochondrial genome assembly and annotation of a morphologically distinct cave cricket specimen systematically collected from Nam Sai Cave, Noen Maprang District, Phitsanulok Province, Thailand. Using high-throughput sequencing and bioinformatics tools, a 16,260 base pair mitochondrial genome was assembled, comprising 37 genes, including 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, with a GC content of 25.57%. The mitochondrial genome exhibits the identical gene arrangement of Orthoptera, suggesting strong conservation across the order. Phylogenetic analysis placed the specimen within the subfamily Aemodogryllinae, revealing a close evolutionary relationship with the genera *Diestrammena*, *Tachycines*, and *Diestramima*, strong molecular and morphological evidence supporting its classification within the tribe Aemodogryllini. These findings provide new genomic data for putative new species in Rhaphidophoridae, offering valuable insights into their phylogenetic relationships, genetic diversity, and adaptations to cave environments. This study lays the foundation for future taxonomic revisions and comparative evolutionary analyses within the family.

Keywords: Aemodogryllini, cave fauna, mitogenome, Orthoptera, phylogenetic analysis, Rhaphidophoridae

INTRODUCTION

Cave crickets, belonging to the suborder Ensifera and family Rhaphidophoridae, are notable for their adaptations to cave habitats, exhibiting distinctive physiological and behavioral traits. The Rhaphidophoridae family is a wingless group composed of ten subfamilies, one of which is extinct, with approximately 1,100 described species (Kim et al. 2024). These crickets typically inhabit dark, moist environments such as caves, underground burrows, and other damp areas like under rocks (Davranoglou et al. 2021). They often emerge at night for feeding or mating (Hegg et al. 2022). Adaptations to these challenging environments include reduced eye pigmentation, as vision is less critical in darkness, and elongated antennae and legs to navigate and locate prey (Richards 1968). Unlike other Ensifera, Rhaphidophoridae lack stridulatory and auditory organs used for acoustic communication (Kim et al. 2024). However, most crickets, including some cave cricket species are known to produce courtship signals by tapping their abdomen or vibrating their body (Stritih and Čokl 2012; Kim et al. 2024; Stritih-Peljhan and Žunič-Kosi 2024).

The subfamily Aemodogryllinae is primarily distributed across the tropical regions of Southeast Asia and the eastern Palaearctic (Gorochov and Storozhenko 2015). Historically, Aemodogryllinae was classified under Rhaphidophorinae until Gorochov reinstated it as a separate subfamily in 1998. The distinguishing features between Aemodogryllinae and Rhaphidophorinae include differences in the structure of the head rostrum, and the absence of styli on the male subgenital plate (Storozenko et al. 2015). Aemodogryllinae comprises two tribes: Aemodogryllini (Jacobson, 1902), and Diestramimini (Gorochov, 1998). Key characteristics of this subfamily encompass the absence of styli on the male subgenital plate (Storozenko et al. 2015), the lack of apical serration on the ventral valvae of the ovipositor (Storozenko et al. 2015). Species within this subfamily are predominantly cave dwellers, with several adapting specifically to subterranean environments (Kurniawan et al. 2022).

Within the subfamily Aemodogryllinae, the tribes Aemodogryllini and Diestramimini exhibit distinct morphological differences. In Aemodogryllini, males possess genitalia characterized by a median semi-sclerotized plate adorned with numerous small denticles, whereas in Diestramimini, the male genitalia are membranous (Gorochov and Storozhenko 2015). Additionally, Diestramimini males exhibit a pronounced posterior process on the seventh abdominal tergite (Gorochov and Storozhenko 2015; Dawwrueng et al. 2016), while Aemodogryllini, the posterior

margin of the seventh tergite lacks a median projection (Storozenko et al. 2015). These morphological distinctions are crucial for accurate taxonomic classification within the Rhaphidophoridae family.

Nam Sai Cave, located in the Noen Maprang District of Phitsanulok, Thailand, was selected for this study due to its known abundant population of cave cricket species, as indicated by previous surveys. This limestone cave serves as a habitat for a diverse range of cave-dwelling organisms, including bats, cave spiders, cave centipedes, cave geckos, and cave crickets (Price 2014; Deharveng et al. 2023). Despite studies of Thai caves, research in this region remains limited, highlighting the importance of making a database of mitochondrial genomes for cave crickets from Thailand. This gap limits our understanding of their evolutionary history and genetic diversity. This survey aims to address this knowledge gap by exploring Nam Sai Cave, particularly focusing on the cave cricket species, marking the first documented investigation of a cave in Phitsanulok.

The examination of mitochondrial genomes is crucial for understanding the evolutionary biology and ecological adaptations of cave crickets. Despite their small size and conserved nature, mitochondrial genomes offer valuable insights into phylogenetics, and molecular evolution (Song et al. 2016). They are also widely used in biogeographical and molecular biological studies (Gong et al. 2021). Since mitochondrial DNA is maternally inherited and remains relatively stable across generations (Sato and Sato 2017; Cao et al. 2024), it serves as an ideal molecular marker for investigating genetic diversity, evolutionary history, and species differentiation within the Rhaphidophoridae family (Wang et al. 2016; Yuan et al. 2018; Priyono et al. 2020; Dowling and Wolff 2023).

This study presents the first complete mitochondrial genome of a cave cricket from Phitsanulok, Thailand. The findings highlight the importance of mitochondrial genome analysis in understanding the genetic diversity, evolutionary relationships, and ecological adaptations of cave crickets. Moreover, this study represents a significant step in filling the knowledge gap in cave cricket biodiversity in Thailand and highlights the importance of further taxonomic and phylogenetic investigations in the region.

MATERIALS AND METHODS

Sample collection, preservation, and DNA extraction

Representative specimens of cave crickets were collected from Nam Sai Cave, Noen Maprang District, Phitsanulok, Thailand, 16°41'26.6"N - 100°40'35.7"E. This site was chosen based on previous surveys indicating a high abundance of cave cricket populations. Specimens (illustrated in Figure 1) were immediately preserved in 75% ethanol and stored at -20°C to maintain tissue integrity for downstream analyses. Morphological identification was performed using key characters including long antennae, elongated legs, large hind legs with "drumstick-shaped" femora, a humpbacked body profile, absence of wings, and a head that is distinctly bent downward. Identification to the tribe level was achieved by comparing these features to established morphological keys. Detailed external morphology was examined under an Olympus SZ11 Zoom Stereo Microscope with a magnification of 10×, and high-resolution digital images were captured using a Nikon D7500 camera. Measurements were taken using a steel ruler with millimeter scale, which provides an approximate precision of ±0.5 mm. Body length was the distance from apex of vertical frons to apex of abdominal tergite, while hind leg length was measured from base to apex of hind leg. All measurements were performed manually on specimens preserved in ethanol. ensuring the ruler was aligned flat against the specimen and minimizing errors due to viewing angle. These measurements were used to supplement morphological identification.

Genomic DNA was extracted specifically from the leg tissues of the cave crickets using the DNeasy Blood & Tissue Kit (Qiagen). To ensure removal of RNA contaminants, RNase A was added during the extraction process.

Sequencing, assembly, annotation, and correction

The purified genomic DNA was shipped to Macrogen (Korea) for sequencing using the Illumina platform. The generated paired-end fastq reads were subjected for downstream analysis. Initially, raw paired-end reads were quality-checked using FastQC Version 0.12.1 (Galaxy Version 0.74+galaxy0) (Brown et al. 2017). Subsequent trimming of low-quality bases and adapter sequences was performed with Trimmomatic version 0.39 (Galaxy Version 0.39+galaxy2) (Bolger et al. 2014), with the following parameters: ILLUMINACLIP: TruSeq3:2:30:10:8, LEADING: 3, TRAILING: 3, SLIDINGWINDOW: 4:15, AVGQUAL, MINLEN: 36.

The high-quality, trimmed reads were then assembled de novo into a draft mitochondrial genome using NOVOplasty version 4.3.1 (Galaxy Version 4.3.1+galaxy0) (Dierckxsens et al. 2017), NOVOplasty was executed using the parameters listed: platform: Illumina, read length: 150 bp, insert size: 300, genome range: 14,000-18,000 bp, k-mer size: 29.

Figure 1. A cave cricket from Nam Sai Cave, Phitsanulok, Thailand, displaying the elongated antennae and limbs characteristic of troglobitic orthopterans

The seed sequence plays an important role in the assembly process. Initially, a *cox1* gene from another cave cricket species available on NCBI was attempted as a seed. However, a circular structure was not obtained from the assembly. Consequently, a seed sequence was generated from our own data by extracting contigs from the NOVOplasty output file, which was then annotated using MITOS version 1.1.1 (Galaxy Version 1.1.1+galaxy0) (Bernt et al. 2013) to identify the *cox1* region. Following the annotation, the *cox1* sequence was isolated, and a BLAST (Altschul et al. 1990) search was performed to confirm its identity with the CDS feature. The confirmed *cox1* sequence was subsequently used as the seed for assembly. The assembled mitochondrial genome was validated for its circular structure and nucleotide composition for feature annotation. The assembled mitochondrial genome was annotated with MITOS version 1.1.1 (Galaxy Version 1.1.1+galaxy0) (Bernt et al. 2013) to predict Coding Sequences (CDS), ribosomal RNAs, and other features. To verify the accuracy of annotated CDS features, the assembly was further aligned against nucleotide sequences using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). tRNA predictions were confirmed using tRNAscan-SE version 2.0 (Chan and Lowe 2019), and all 22 tRNA genes were manually inspected to ensure the presence of the appropriate anticodons corresponding to their respective amino acids.

The finalized annotated mitochondrial genome of this putative cave cricket species (Rhaphidophoridae) was submitted to the National Center for Biotechnology Information (NCBI) GenBank database (Benson et al. 2013) under accession number OR865114.1. A graphical representation of the mitochondrial genome was generated using the OrganellarGenomeDRAW (OGDRAW) server version 1.3.1 (Greiner et al. 2019) based on the annotated GenBank file.

Phylogenetic tree reconstruction

Phylogenetic tree reconstruction of the evolutionary relationships among various species within the Rhaphidophoridae family, focusing on the cave cricket mitochondrial genome. The process began with the collection of mitochondrial genome data of cave cricket species available on NCBI, a total of 32 species. These included all Rhaphidophoridae species mitochondrial genomes available at the time of the analysis, identified based on BLAST searches. Subsequently, these sequences were aligned to identify homologous regions with MUSCLE alignment (Edgar 2004). The best model, general time reversible model with gamma distributed with invariant sites (GTR+I+G), was identified using jModelTest version 2.1.10 (Darriba et al. 2012), based on the lowest BIC score (451102.133688). The model parameters were as follows: gamma shape = 0.4820, proportion invariant = 0.2420, substitution model: rate AC = 1.2285, rate AG = 6.0607, rate AT = 1.7505, rate CG = 3.1965, rate CT = 14.5536, and rate GT = 1.0000. Next, the parameters were set, and the phylogenetic tree was generated using BEAST software version 2.6.7, using Markov chain Monte Carlo for 10 million generations (Drummond and Rambaut 2007). Figtree version 1.4.4 (Rambaut 2007) was used to visualize the phylogenetic tree.

RESULTS AND DISCUSSION

Cave cricket morphology

The cave cricket specimen collected from Nam Sai Cave, Noen Maprang District, Phitsanulok, Thailand, exhibits morphological characteristics typical of the family Rhaphidophoridae. The observed individuals had an average body length of approximately 1.2-1.5 cm, body small to medium size, with notably elongated hind legs measuring around 4.5-5 cm. Hind tibiae with 30-36 spines on both sides. Hind basitarsus with one apical spine on dorsal surface. The body coloration was primarily brown, with fine setae distributed across the exoskeleton, which may contribute to sensory perception in low-light environments. Morphological examination of the male specimens revealed that the posterior margin of the seventh tergite lacked a long median projection, a key trait distinguishing member of the tribe Aemodogryllini from Diestramimini (Figure 2). These findings support the classification of the cave cricket within the Aemodogryllini tribe, aligning with previously established taxonomic keys (Storozenko et al. 2015). Further comparative morphological analysis with related taxa is necessary to confirm species-level identification due to the insufficient morphological characteristics available in this study, definitive species identification remains inconclusive.

Cave cricket mitochondrial genome

The complete mitochondrial genome of the Rhaphidophoridae specimen was successfully assembled, yielding a circular genome of 16,260 base pairs, with an average sequencing coverage of 14,182×. The overall GC content was determined to be 25.57%, consistent with previously sequenced mitochondrial genomes within Rhaphidophoridae. Mitochondrial genome was submitted to the National Center for Biotechnology Information (NCBI), Accession number: OR865114. Gene annotation revealed a total of 37 genes, including 13 protein-coding genes (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad5*, *nad4*, *nad4l*, *nad6*, *cob*, and *nad1*), 22 transfer RNA (tRNA) genes (*Ile*, *Gln*, *Met*, *Trp*, *Cys*, *Tyr*, *Leu-2*, *Lys*, *Asp*, *Gly*, *Ala*, *Arg*, *Glu*, *Ser-1*, *Asn*, *Phe*, *His*, *Thr*, *Pro*, *Ser-2*, *Leu-1*, and *Val*), and 2 ribosomal RNA (rRNA) genes (*L-rRNA* and *S-rRNA*). This gene arrangement is identical to that found in most orthopteran mitochondrial genomes, suggesting strong conservation across the order. The details of all mitochondrial genome annotated genes are summarized in Table 1, and the complete mitochondrial genome map is illustrated in Figure 3.

Phylogenetic analysis and taxonomic placement

To assess the evolutionary placement of the cave cricket species, a phylogenetic tree was constructed using complete mitochondrial genome sequences from Rhaphidophoridae species. The resulting Bayesian phylogenetic tree (Figure 4) provides insights into the evolutionary relationships

among cave crickets and their closest relatives. Phylogenetic analysis revealed that the cave cricket species from Nam Sai Cave clusters closely with members of the genera *Diestrammena*, *Tachycines*, and *Diestramima*, which are part of the subfamily Aemodogryllinae. Within this subfamily, species are further classified into two primary tribes: Aemodogryllini and Diestramimini. The close phylogenetic association of the studied species with *Diestrammena* and *Tachycines* strongly suggests that it belongs to the tribe Aemodogryllini. In contrast, species within the genus *Diestramima* are positioned within Diestramimini, a tribe predominantly distributed in southern China and Southeast Asia (Zhu et al. 2023).

Further analysis of tribal relationships highlights the evolutionary divergence within Aemodogryllinae. The clustering of *Diestrammena* sp. and *Tachycines* sp. suggests a shared evolutionary ancestry, reinforcing the distinction between Aemodogryllini and Diestramimini. Despite the tree lacking divergence support values, the relationships of cave cricket species within Aemodogryllinae are consistent with Dorji et al. (2025), supporting the preliminary recognition of tribal-level groupings within the subfamily. Outside this subfamily, species such as *Troglophilus neglectus* (Krauss, 1879) (Troglophilinae, Troglophilini), *Ceuthophilus* sp. (Ceuthophilinae, Ceuthophilini), and *Rhaphidophora quadrispina* (Liu & Bian, 2021) and *R. duxiu* (Lu & Bian, 2022) (Rhaphidophorinae, Rhaphidophorini) occupy distinct phylogenetic positions. A previous study indicated that the subfamily Troglophilinae evolved from Rhaphidophorinae (Allegrucci and Sbordoni 2019). Outside Aemodogryllinae, species from Macropathinae (including *Dendroplectron aucklandensis* (Richards, 1964), *Heteromallus* sp., *Insulanoplectron spinosum* (Richards, 1970), and others) form a separate clade, supporting the hypothesis that Macropathinae represents a distinct evolutionary lineage distributed across Australia, New Zealand, South America, and southern Africa. The observed separation of Macropathinae from other subfamilies highlights biogeographical influences on the evolutionary history of Rhaphidophoridae. The observed clustering aligns with morphological traits, reinforcing taxonomic classification.

Discussion

The integration of morphological and molecular analyses in this study provides a comprehensive understanding of the cave cricket specimen from Nam Sai Cave. Morphologically, the absence of a long median projection on the seventh tergite in males is a defining characteristic of the tribe Aemodogryllini (Storozenko et al. 2015). This morphological trait is consistent with our phylogenetic findings, which place the specimen in close relation to genera such as *Diestrammena* and *Tachycines*, both members of Aemodogryllini. However, despite these close phylogenetic relationships, several morphological and molecular characteristics suggest this specimen represents a potentially undescribed species within the tribe. Additionally, the presence of fine setae distributed across the body may be an adaptation to the low-light conditions typical of cave environments. These mechanosensory structures potentially enhance environmental perception where vision is limited, representing troglomorphic adaptation common among obligate cave-dwellers.

Table 1. Mitochondrial genome annotation summary of the Rhaphidophoridae species

Gene	Direction	Location	Size (bp)	Start codon	Stop codon
trnI(gat)	+	517-583	67	-	-
trnQ(ttg)	-	581-649	69	-	-
trnM(cat)	+	662-730	69	-	-
nad2	+	732-1760	1029	ATG	TAG
trnW(tca)	+	1766-1832	67	-	-
trnC(gca)	-	1825-1892	68	-	-
trnY(gta)	-	1900-1964	65	-	-
cox1	+	1957-3496	1540	ATT	ATT
trnL2(taa)	+	3497-3562	66	-	-
cox2	+	3563-4253	691	ATG	CAT
trnK(ctt)	+	4254-4322	69	-	-
trnD(gtc)	+	4324-4389	67	-	-
atp8	+	4390-4548	159	ATT	TAA
atp6	+	4542-5219	678	ATG	TAA
cox3	+	5219-6007	789	ATG	TAA
trnG(tcc)	+	6016-6082	67	-	-
nad3	+	6083-6436	354	ATT	TAG
trnA(tgc)	+	6435-6499	65	-	-
trnR(tcg)	+	6502-6565	64	-	-
trnN(gtt)	+	6570-6626	67	-	-
trnS1(gct)	+	6637-6704	68	-	-
trnE(ttc)	+	6710-6780	71	-	-
trnF(gaa)	-	6783-6849	67	-	-
nad5	-	6850-8585	1736	ATG	TTA
trnH(gtg)	-	8587-8653	67	-	-
nad4	-	8654-9992	1339	ATG	TGT
nad4l	-	9986-10279	294	ATG	TAA
trnT(tgt)	+	10282-10345	64	-	-
trnP(tgg)	-	10345-10410	66	-	-
nad6	+	10412-10939	528	ATA	TAA
cob	+	10939-12075	1137	ATG	TAA
trnS2(tga)	+	12075-12142	68	-	-
nad1	-	12174-13124	951	TTG	TAA
trnL1(tag)	-	13125-13191	67	-	-
rrnL	-	13168-14539	1372	-	-
trnV(tac)	-	14538-14608	71	-	-
rrnS	-	14619-15418	800	-	-

Figure 2. The seventh tergite of the male without a long median projection

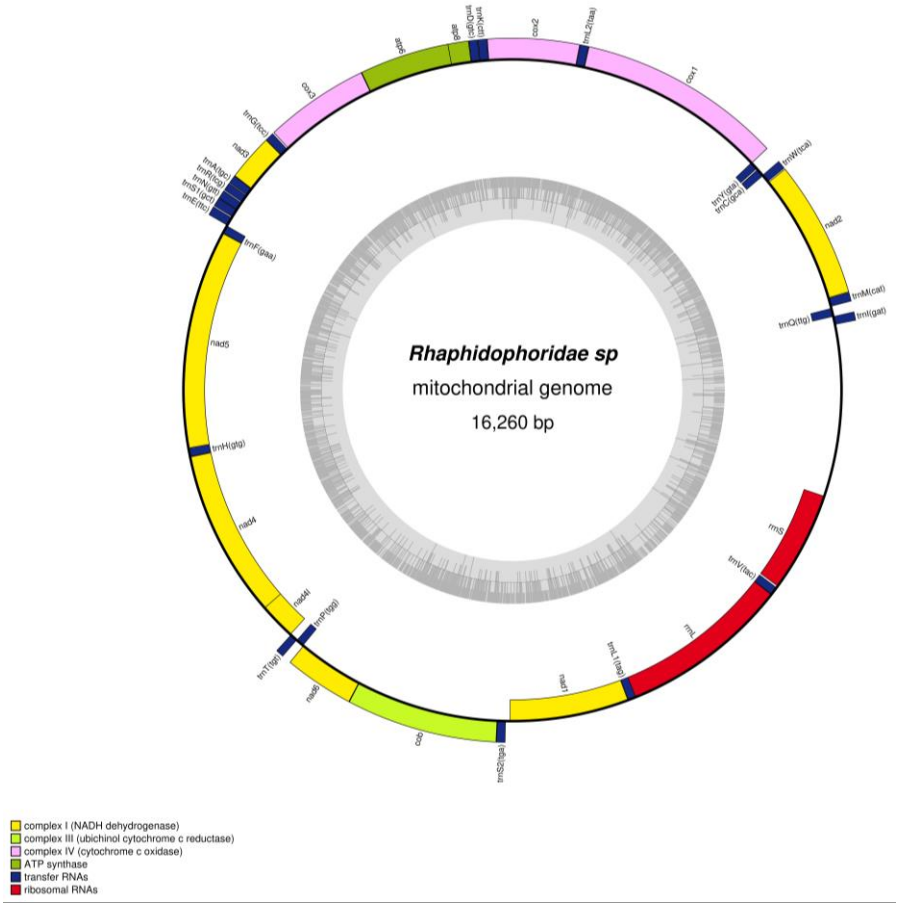

Figure 3. Complete mitochondrial genome organization of cave cricket Rhaphidophoridae species from Nam Sai Cave, Thailand. Circular representation of the 16,260 bp mitochondrial genome showing the spatial arrangement and transcriptional orientation of all 37 genes, including 13 protein-coding genes (yellow), 22 tRNA genes (blue), and 2 rRNA genes (red), following the conserved arthropod gene organization pattern

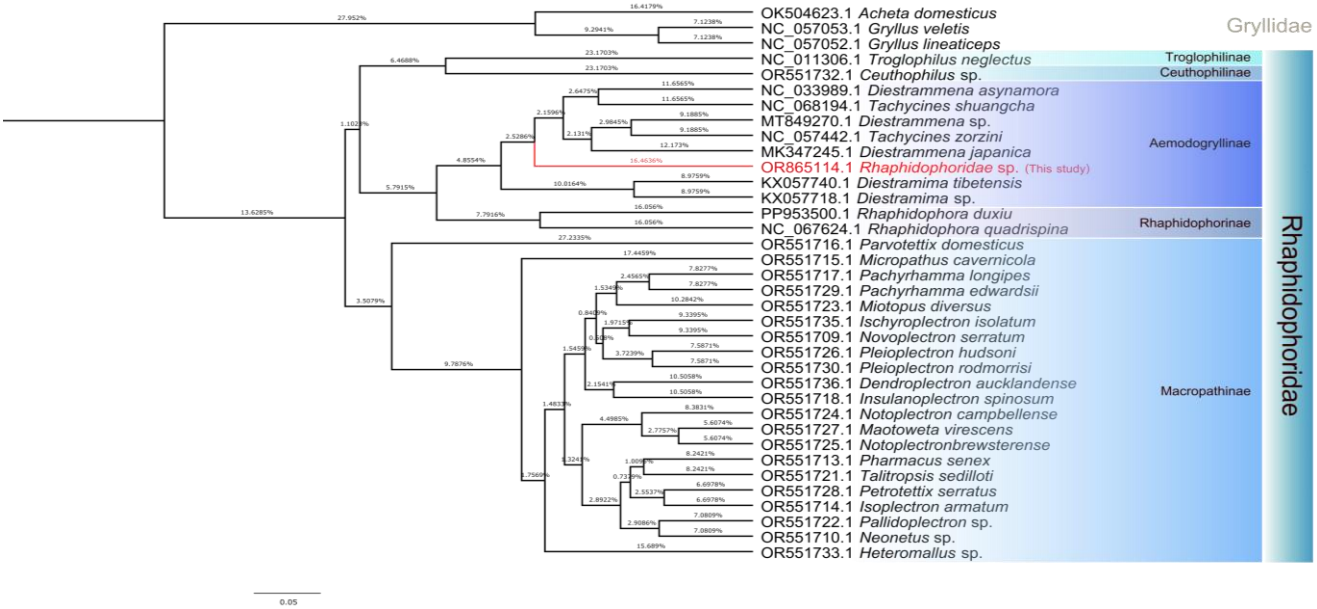

Figure 4. Phylogenetic tree constructed using Bayesian Evolutionary Analysis Sampling (BEAST) software based on 33 mitochondrial genomes of Rhaphidophoridae species. Crickets from Gryllidae (*Acheta domesticus* (Linnaeus, 1758), *Gryllus lineaticeps* (Stål, 1861), and *Gryllus veletis* (Alexander & Bigelow, 1960)) were included as an outgroup

Other cave cricket species, such as *Troglophilus neglectus*, have also shown the adaptation in the thorax for cave habitats (Leubner et al. 2016). These morphological adaptations may be associated with the previously mentioned environmental limitations, along with the isolation characteristic of subterranean ecosystems. Also, the darkness is considered to be the major factor and the most conspicuous feature impacting that driving the adaptation, especially of behavior, to the subterranean environments (Culver and Pipan 2019; Lunghi et al. 2024). Evolutionary adaptations are observed not only in cave crickets but also in other cave-dwelling organisms, including *Astroblepus pholeter* (Collette, 1962) (cavefish) (Espinasa et al. 2018) and *Proteus anguinus* (Laurenti, 1768) (olm) (Tesařová et al. 2022). Our integration of morphological and molecular data is similar to Seesamut et al. (2023), they report the first record of cavernicolous land leech (*Sinospelaeobdella cavatuses*) in Thailand using morphological and molecular data. Similarly, Ahmed et al. (2025) utilized both datasets to revise the taxonomy of Tunisian nasal leeches (*Limnatis nilotica* (Savigny, 1822)), revealing a second *Limnatis* species in North Africa. Moreover, a recent study by Srisonchai et al. (2025) combined both datasets to confirm the taxonomic status of dragon millipedes in Thailand. However, definitive species-level identification requires additional comparative genomic data and expanded morphological sampling to navigate the complex taxonomic landscape characteristic of Rhaphidophoridae systematics.

The mitochondrial genome analysis revealed a circular genome of 16,260 base pairs, encompassing the typical 37 genes found in orthopteran species. The observed GC content of 25.57% is consistent with previously reported values within Rhaphidophoridae, and reflects a conserved genomic structure across the family that typically shows A+T richness which is a common trait in insect mitochondrial genomes (Shokolenko and Alexeyev 2022). Similarly, the mitochondrial genome of *Tachycines* (*Gymnaeta*) *zorzini* (Rampini & Di Russo, 2008) has given insights into the phylogenetic position of this species among other Ensifera. Phylogenetic analysis revealed that *Tachycines* (*Gymnaeta*) *zorzini* is closely related to *Tachycines* (*Tachycines*) *minor* (Chopard, 1963), and that the genera *Tachycines* and *Diestrammena* are monophyletic (Wang et al. 2021). In addition, the complete mitochondrial genome of *R. duxiu* was recently sequenced, revealing a circular genome of 15,898 base pairs, comprising 13 protein-coding genes, 2 ribosomal RNA genes, and 22 transfer RNA genes. This genomic information is identical in gene content to *R. quadrispina* and provides a foundation for further studies on genomic resources and genetic relationships within the Rhaphidophoridae family (Yu et al. 2025). This conservation suggests that mitochondrial genomes can serve as reliable molecular markers for phylogenetic studies within this group. Our phylogenetic findings are in agreement with recent studies that have utilized mitochondrial and nuclear loci to elucidate relationships within Rhaphidophoridae. Understanding the genetic diversity and evolutionary relationships of cave crickets is particularly crucial given their ecological significance as keystone species in subterranean ecosystems.

The phylogenetic analysis indicates that the cave cricket collected from Nam Sai Cave is closely related to cave crickets of the genera *Diestrammena* and *Tachycines*, both of which are recognized within the tribe Aemodogryllini. However, species from the genus *Diestramima* belong to the tribe Diestramimini differs from Aemodogryllini in both molecular characteristics and specific morphological traits, such as the presence of a long median projection on the seventh tergite in both sexes. This separation highlights the evolutionary divergence that has occurred between these two closely related tribes. The divergence may be influenced by geographic isolation among cave systems and habitat specialization, leading to speciation (Zhu et al. 2022). Comprehensive phylogeny of cave crickets highlighted the monophyly of major subfamilies and tribes, supporting the taxonomic framework employed in our study (Kim et al. 2024). Additionally, research on the genus *Diestramima* has demonstrated the utility of mitochondrial markers in resolving species boundaries and understanding biogeographic patterns (Zhu et al. 2023).

Phylogenetic analysis helps clarify the geographic patterns that influence evolutionary relationships. The clade of Southeast Asian species like Aemodogryllini with the cave cricket collected from Nam Sai Cave indicates that cave crickets tend to have increased potential for isolation in subterranean ecosystems (Castro-Souza et al. 2023). Macropathinae, the distinct clade mostly found in Australia, New Zealand, South America, and southern Africa. Australian taxa showing paraphyly and close relationships to New Zealand and South American Macropathinae lineages (Beasley-Hall et al. 2018). Cave cricket species generally inhabit cave-like environments characterized by minimal variation in temperature or moisture (Kozel et al. 2019; Souza-Silva et al. 2021; Junta et al. 2025). This suggests a limited dispersal capability and specific habitat requirements have led to distinct evolutionary patterns (Allegrucci et al. 2017; Davranoglou et al. 2021; Castro-Souza et al. 2023). As a result, cave crickets in Aemodogryllinae and Macropathinae were separated by geographical structuring. Moreover, cave crickets play as a keystone in cave ecosystems and are often used as bioindicators of environmental conditions (Kurniawan et al. 2022). Their abundance indicates the overall health of their habitats (Kurniawan et al. 2022). The conservation of cave environments is critical for preserving the ecological conditions necessary to sustain cave cricket populations and subterranean communities (Weckstein et al. 2016; Castro-Souza et al. 2023). Moreover, unregulated tourism presents notable threats to cave-dwelling organisms through microclimate disruption mechanisms that can destabilize the environmental conditions characteristic of cave systems and negatively impact sensitive species dependent on stable subterranean habitats (Sagot and Chaverri 2015). The taxonomic uncertainty highlighted by our findings underscores the critical importance of comprehensive biodiversity surveys in cave systems, particularly given that cave crickets serve as bioindicators of ecosystem health and their conservation status directly reflects the integrity of subterranean habitats.

The integration of morphological and molecular data in our study not only reinforces the classification of the Nam Sai Cave specimen within Aemodogryllini but also highlights the importance of using multiple lines of evidence in taxonomic studies. This approach enhances the robustness of taxonomic conclusions and provides a more comprehensive understanding of evolutionary relationships. However, our study could not identify the species of the cave crickets, underscoring the need for further taxonomic revision and more comparative datasets. Given the ecological significance of cave crickets in subterranean ecosystems, such studies are essential for informing conservation strategies and understanding the evolutionary processes that shape biodiversity in these unique habitats. Future research aims to broadly identify morphological characteristics and mitochondrial genomes to improve phylogenetic resolution and help identify species precisely.

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