MITOCHONDRIAL DNA PART B: RESOURCES
2024, VOL. 9, NO. 4, 457–460
https://doi.org/10.1080/23802359.2024.2316064

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MITOGENOME REPORT

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The complete mitochondrial genome of *Phymateus saxosus* (Coquerel, 1861) (Orthoptera: Pyrgomorphidae) and phylogenetic analysis

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ABSTRACT

Phymateus saxosus is a member of the family Pyrgomorphidae, Orthoptera. In this study, the complete mitochondrial genome (mitogenome) of *P. saxosus* was determined and analyzed. Assembled mitogenome sequence of *P. saxosus* is 15,672 bp in size, containing 37 genes and a control region. The gene orientation and arrangement of *P. saxosus* are identical to other species in the Pyrgomorphoidea family. The overall nucleotide composition is as follows: A (43.6%) > T (30.2%) > C (16.1%) > G (10.1%). Phylogenetic analysis suggested that *P. saxosus* forms sister groups with *P. morbillosus*, and the monophyly of Pyrgomorphidae is supported. In general, this study provided valuable genetic information for *P. saxosus* and explored the phylogenetic relationships in the family Pyrgomorphidae.

ARTICLE HISTORY
Received 20 December 2023
Accepted 3 February 2024

KEYWORDS
Mitochondrial genome; phylogeney; pyrgomorphidae; Caelifera

1. Introduction

There is only one family in Pyrgomorphoidea (Orthoptera: Caelifera), Pyrgomorphidae. Pyrgomorphidae is one of the most charismatic grasshopper families, well known for their vibrant body color and conspicuous sculpting patterns on pronotum, and often featured in display collections of large and showy insects (Mariño-Pérez and Song 2018). The family currently includes 487 valid species, but the complete mitogenome sequences of only 14 Pyrgomorphidae species are available in the NCBI database (https://www.ncbi.nlm.nih.gov/). At present, many studies have been conducted on the phylogenetic relationships of Orthoptera insects. However, only a few have included Pyrgomorphidae (Cameron 2014; Chang et al. 2020a). There are still unresolved issues in the phylogeny of Pyrgomorphidae, requiring additional data for further analysis (Mariño-Pérez and Song 2019).

Phymateus belongs to Pyrgomorphinae of Pyrgomorphidae, of which 12 species are recorded in Orthoptera Species File Online (http://orthoptera.speciesfile.org/) (Cigliano et al. 2023). *Phymateus saxosus* (Coquerel, 1861), a large grasshopper of *Phymateus*, is known for being inedible in Madagascar (Braud et al. 2014). The adults of this species have aposematic coloration, with spiny or protruding anterior pectoral backplane and yellow spots on their forewings (Figure 1). Moreover, they produce an unpleasant smell when disturbed (Van Itterbeeck et al. 2019). In this study, we determined the first complete mitogenome of *P. saxosus* and constructed a phylogenetic tree of Caelifera to provide data support and molecular evidence for the phylogenetic relationship of *P. saxosus* with other species in Pyrgomorphidae.

Figure 1. Photography of *Phymateus saxosus* taken by Huihui Chang, in July, 2023.

2. Materials and methods

2.1. Sample collection and DNA extraction

The samples of *P. saxosus* were collected from Moramanga of Madagascar (18°52′37″S, 48°27′37″E) on 1 February 2021. The specimen was preserved in 99.7% ethanol and stored in a 4 °C freezer at Henan University of Urban Construction

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2024.2316064.

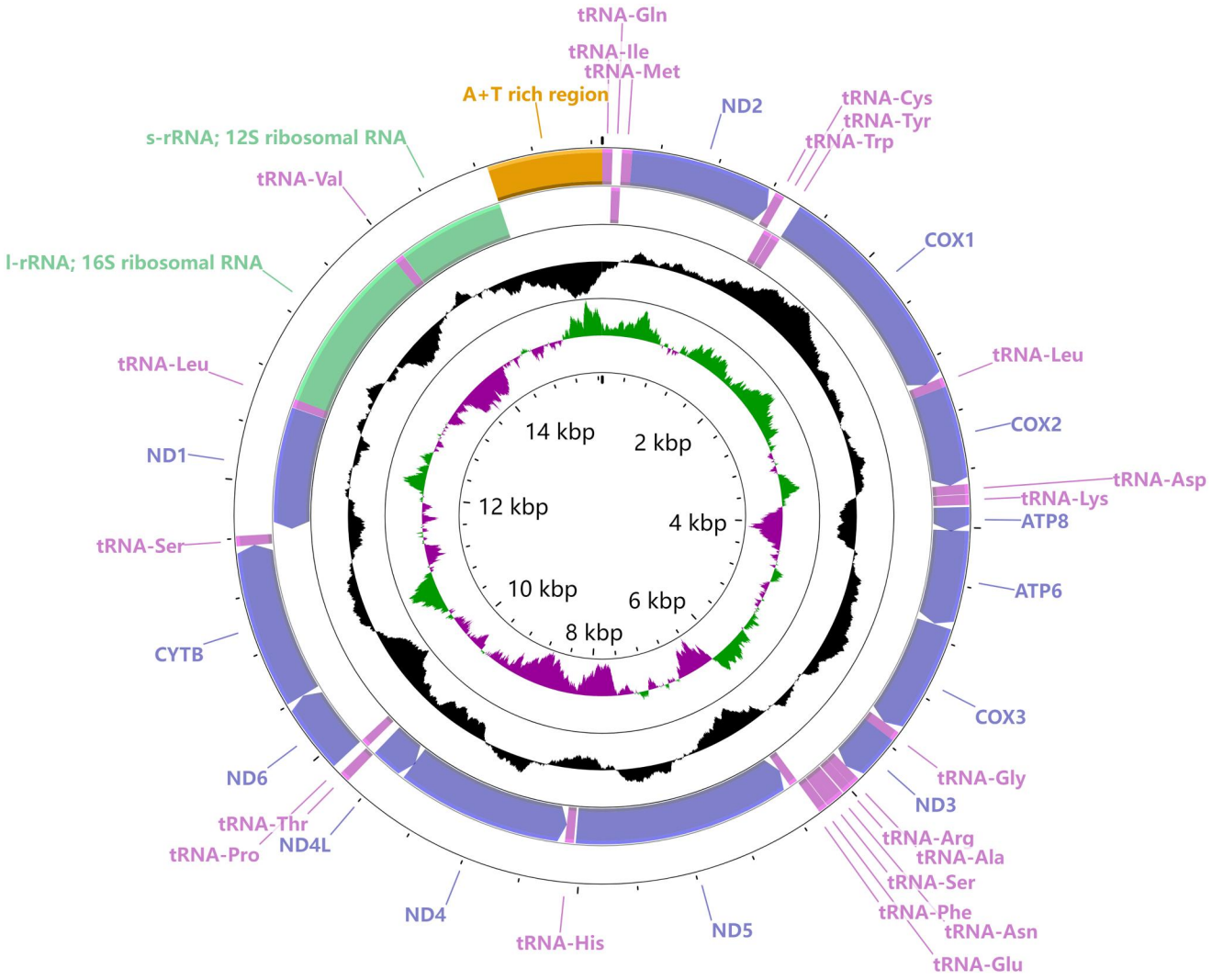

Figure 2. Gene map of the mitochondrial genome of *Phymateus saxosus*. Plots of GC content and skew used a window size of 500 and reflect GC content/skew scores on a scale of 0 to 1 using a baseline of 0.5. Positive and negative skew are indicated by values above and below the midpoint respectively.

(https://www.huuc.edu.cn/, contact person: Huihui Chang, changhuihui@huuc.edu.cn) with the voucher ID H005. Total genomic DNA was extracted from the muscle tissue by a DNeasy Blood and Tissue Kit ((50)-QIAGEN 69504), and then stored at −20 °C.

2.2. Genome sequencing, assembly and annotation

An Illumina HiSeq 2500 system was used to sequence the DNA of *P. saxosus* with a 150 bp read length. DNA library construction and sequencing were conducted by the Biomarker Company. The *COX1* gene sequence was extracted from mitochondrial genome of *Mekongiella xizangensis* (GenBank accession number: NC_014451) as the seed sequence, and the new mitochondrial genome was assembled by NOVOPlasty v4.3 (Dierckxsens et al. 2017). The 40,587 reads were mapped in Geneious Prime (Kearse et al. 2012) (available from http://www.geneious.com) to check the assembled mitogenome, yielding a coverage of 540× (Figure S1). RNAs were identified by MITOS2 (Donath et al. 2019) (http://mitos.bioinf.uni-leipzig.de/). The other genes were determined in Geneious Prime by comparing them to the reference mitogenome and other closely related mitogenomes, and then checked manually. The genome structure was mapped using Proksee (Grant et al. 2023) (https://proksee.ca/).

2.3. Phylogenetic analysis

A phylogenetic tree was created based on Maximum-Likelihood (ML) using 35 Caelifera mitogenome sequences, which included 14 complete mitogenomes from Pyrgomorphidae (one of which was newly discovered) and 20 complete mitogenomes from seven other families. The dataset was made up of 13 PCGs and 2 rRNAs. The individual gene data sets were first aligned using MAFFT v7.313 (Katoh et al. 2009) and then concatenated by PhyloSuite v1.2.2 (Zhang et al. 2020). Modelfinder (Kalyaanamoorthy et al. 2017) was used to determine the optimal partitioning models. Finally, IQ-tree v1.6.8 (Nguyen et al. 2015) was utilized to establish the ML tree with 5000 bootstrap values.

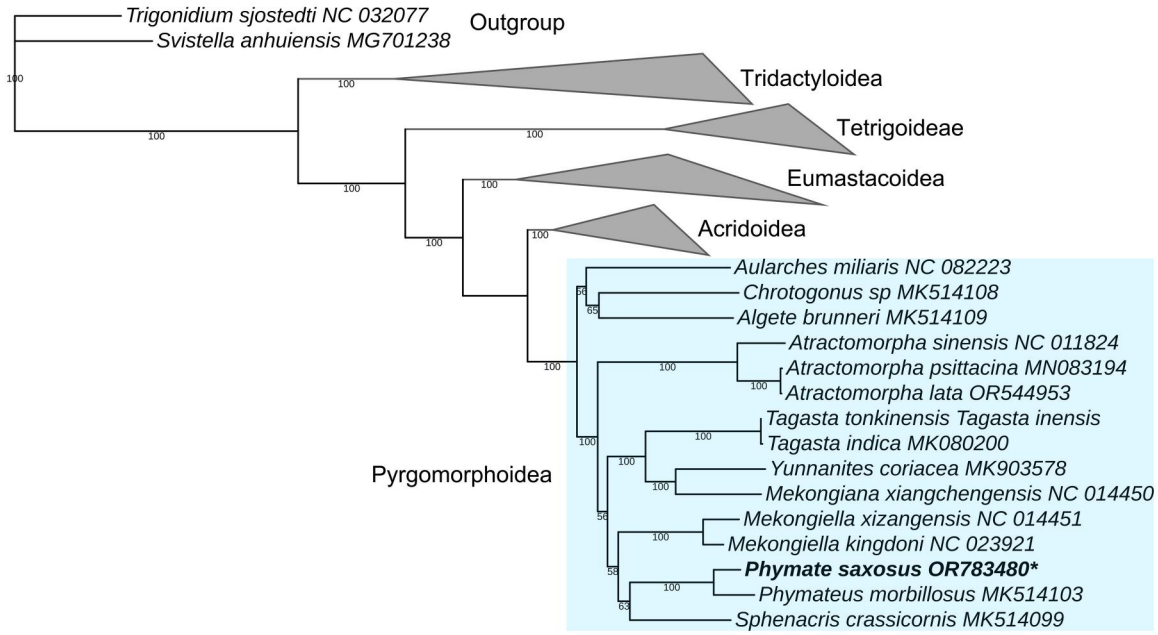

Figure 3. Phylogenetic analysis based on Maximum-Likelihood of 35 Caelifera mitogenome sequences, including the newly sequenced *Phymateus saxosus*, using 13 protein-coding genes and 2 rRNAs. Nodal support values indicate the Maximum-Likelihood bootstrap support value (BP). The newly sequenced *P. saxosus* is highlighted by an asterisk and bold. Of the four collapsed nodes, Tridactyloidea includes *Mirhipipteryx andensis* NC 028065 and *Ellipes minuta* NC 014488, Tetrigoideae includes *Tetrix japonica* NC 018543 and *Teredorus nigropennis* MN938922, Eumastacoidea includes *Pseudothericles compressifrons* NC 0280611 and *Pielomastax zhengi* NC 016182 and Acridoidea includes *Filchnerella helanshanensis* NC 020329, *F. beicki* NC 024923, *Acrida cinerea* NC 014887, *A. willemsei* NC 011303, *Locusta migratoria migratoria* NC 011119, *L. migratoria manilensis* NC 014891, *Gomphocerus sibiricus tibetanus* NC 015478, *G. sibiricus* NC 021103, *Peripolus nepalensis* NC 029135, *Calliptamus italicus* NC 011305, *Tonkinacris sinensis* NC 032716, *Sinopodisma kelloggii* NC 071954, *Oxya hyla* NC 032076 and *O. chinensis* NC 010219.

3. Results

3.1. Characteristics of P. saxosus mitogenome

The complete mitogenome of *P. saxosus* (OR783480) is 15,672 bp in length, with 43.6% A, 30.2% T, 16.1% C and 10.1% G (Figure 2). The genome contains 13 PCGs, 22 tRNAs, two rRNAs and a non-coding control region (A + T rich region, D-loop). Out of the 37 genes, 23 are located on the J-strand, including 9 PCGs (*COX1*, *COX2*, *COX3*, *ATP6*, *ATP8*, *CYTB*, *ND2*, *ND3*, *ND6*) and 14 tRNAs (*trnI*, *trnM*, *trnW*, $trnL^{UUR}$, *trnD*, *trnK*, *trnG*, *trnA*, *trnR*, *trnN*, $trnS^{AGN}$, *trnE*, *trnT*, $trnS^{UCN}$), and the remaining 14 are located on the N-strand (Figure S2). The 13 PCGs start with typical ATN initiation codons (one with ATT, two with ATA, two with ATC and seven with ATG), except for *COX1* which begins with CCG. All of the PCGs end with the typical stop codons except for *COX3* which uses T as an incomplete termination codon. The 16S ribosomal RNA (l-rRNA) and 12S ribosomal RNA (s-rRNA) in this genome are 1,311 bp and 832 bp in size, respectively. Additionally, there are 22 transfer RNAs (tRNAs) in this genome which range in size from 64 bp to 72 bp.

3.2. Phylogenetic relationships

The phylogenetic results showed that Pyrgomorphoidea form sister groups with Acridoidea, which diverge later than the other families of Caelifera. Pyrgomorphoidea (only one family, Pyrgomorphidae) is monophyletic and is divided into two clades (Figure 3), with the smaller branch including *Aularches miliaris* (NC 082223), *Chrotogonus* sp. (MK514108) and *Algete brunneri* (MK514109) and the other species cluster into a relatively large branch. In this larger clade, three species of *Atractomorpha* group together and form sister groups with the remaining clades. *Yunnanites*, *Tagasta* and *Mekongiana* cluster into a smaller branch. *P. saxosus* forms sister groups with *Phymateus morbillosus* (MK514103). Orthacridinae (*Sphenacris crassicornis*, MK514099) and *Phymateus* form sister groups and cluster together with species of *Mekongiella*. The monophyly of Pyrgomorphinae cannot be supported, and since only one mitogenome of one species is available in Orthacridinae, the monophyly cannot be tested.

4. Discussion and conclusion

We reported the first complete mitogenome sequencing of *P. saxosus* by high-throughput sequencing and assembly. In the mitogenome of *P. saxosus*, which is 15,672 bp, the orientation and gene order of all 37 genes are identical to other Pyrgomorphoidea species (Mariño-Pérez and Song 2019). The presence of atypical initiation codons and incomplete stop codons in PCGs has also been reported in other Orthoptera species (Qiu et al. 2020; Li et al. 2021, 2022; Zhang et al. 2023).

Phylogenetic analysis showed that the divergence time of Pyrgomorphoidea was later than that of related species of Eumastacoidea, Tetrigoidea and Tridactyloidea, which is consistent with the results of previous studies (Chang et al. 2020a, 2020b). The close relationship between Acridoidea and Pyrgomorphoidea was supported in this study and other published results (Chang et al. 2020a, 2020b; Mao et al. 2020; Qian et al. 2021).

The monophyly of Pyrgomorphidae (Pyrgomorphoidea) is supported (Mariño-Pérez and Song 2019; Chang et al. 2020a, 2020b; Qian et al. 2021), but neither morphological nor

molecular data support the monophyly of Pyrgomorphinae (Mariño-Pérez and Song 2018, 2019), which consistent with the results of this study. The phylogenetic results showed that *P. saxosus* is closely related to *P. morbillosus* than to other species of Pyrgomorphoidea. The relationships between genera within Pyrgomorphidae varied widely among different studies, possibly due to the lack of data available for analysis (Mariño-Pérez and Song 2018, 2019; Chang et al. 2020b), and the relationship between *Phymateus* and other genera needs further analysis.

Ethical approval

This study does not need ethical approval or permissions to collect, handling, and transport of the samples.

Author contributions

HC and ZX designed the study. HC carried out the experiments and drafted the manuscript. HC and XL analyzed the data. XL and ZX modified the final manuscript. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Research and Monitoring Project of Baigui Lake National Wetland Park (Project code: SK202002) and Detection of zooplankton diversity and assessment of water quality ecological health in the wetlands of the Henan section of the Yellow River Basin based on DNA composite barcode technology (Project code: 24B180001).

Data availability statement

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov under the accession number OR783480. The associated BioProject, Bio-Sample and SRA numbers are PRJNA1040470, SAMN38260993 and SRR26909980, respectively.

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