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MITOGENOME REPORT

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The complete mitochondrial genome of *Sigara lateralis* (Leach, 1817) (Nepomorpha: Corixidae)

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ABSTRACT

The *Sigara lateralis* (Leach, 1817) is a small aquatic insect belonging to the family Corixidae. The study aims to reveal the genomic data of the mitochondrial genome of *S. lateralis*. The length of its circular mitochondrial genome is 15,725 bp long with an A + T bias (75.96%). The mitogenome comprises 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA genes, and two rRNA genes. The phylogenetic analyses showed that the *S. lateralis* is the closest to *S. septemlineata*. These findings will help the conservation of Corixidae from the perspective of genetic evolution.

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Sigara lateralis; (Leach, 1817); complete mitochondrial genome; phylogenetic relationship

Introduction

Sigara lateralis (Leach, 1817) is a small aquatic bug that can adapt to various environments, including polluted, eutrophic, and even muddy waters (Teyrovsky 1960; Aukema et al. 2002; Huxley 2003). They feed on aquatic insect carcasses and larvae of small Crustacea (Murillo and Recasens 1986). *S. lateralis* belongs to the family Corixidae, which was known as water boatmen and distributed worldwide (Nowińska et al. 2023). The superfamily Corixoidea, which has more than 600 extant species, is one of the largest superfamilies of Nepomorpha (Ye et al. 2023). No complete mitochondrial genome of Corixidae species has been described yet, except

Figure 1. The image of *Sigara lateralis*, 1817. This photograph was taken by Mengjiao Liu with a stereomicroscope (OLYMPUS SZ61) and digital camera.

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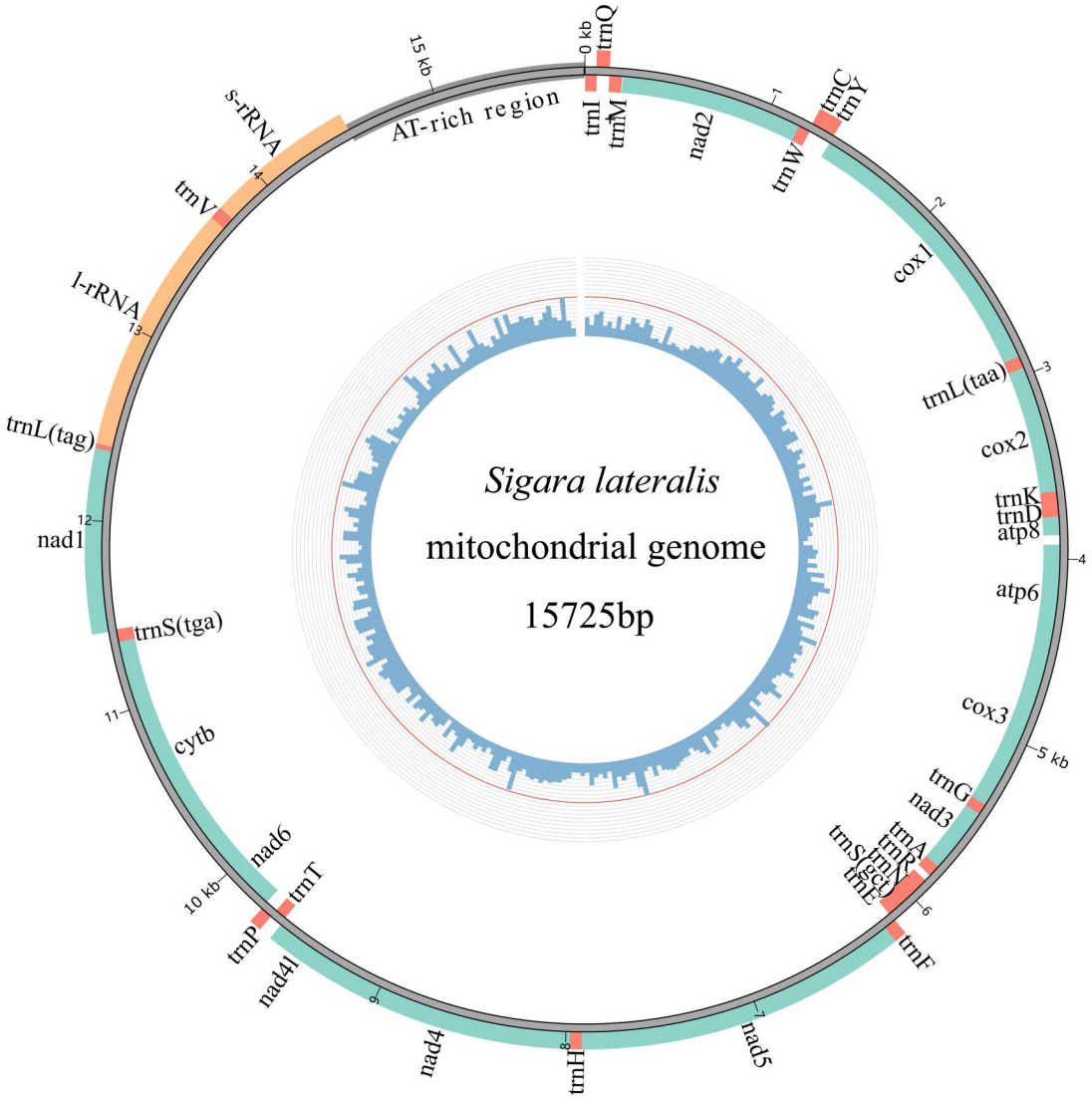

Figure 2. The circular structure of the mitochondrial genome of *Sigara lateralis*. The inner circle represents GC content, the outer circle shows the gene features, gray for at-rich region, orange for rRNA, red for tRNA, and green for CDS. Genes inside the outer circle (on the J chain) are transcribed clockwise, while those outside (on the N chain) are transcribed counter-clockwise.

for the partial mitochondrial genome of *S. septemlineata*. The mitogenome of *S. lateralis* was sequenced and characterized to provide genomic information and data for Corixidae phylogeny.

Materials and methods

The samples (Figure 1) were collected from Cha Khan Nur, Wushen Zhao, Ordos City, Inner Mongolia, China (39°10′23.31″N, 109°04′18.51″E) in the summer of 2021. The samples were identified as *S. lateralis* by their black claws on posterior legs in mature adults and dark areas of hemielytron less extensive than light areas (Savage and Swift 1997). The specimens were deposited in anhydrous ethanol at the Museum of Baotou Teachers College under voucher number no. BA 840066 (Yunpeng Liu, 472426573@qq.com).

Total DNA was extracted using the TIANamp Genomic DNA Kit, and its concentration was determined by 1% agarose gel electrophoresis. The whole genome shotgun method was used to construct the library, and then the paired-end libraries were sequenced on an Illumina HiSeq platform (San Diego, CA) with an insert size of 250 bp. MITObim 1.9 (Hahn et al. 2013) was used to assemble the circular genome because the assembled genome by MITObim was longer than those by other tools (including SOAPdenovo, GetOrganelle, and MitoZ) and had overlapped sequences at both ends. The annotation module in MitoZ (Meng et al. 2019) was used to annotate the assembled mitogenome, and the Circos v.0.69 (Krzywinski et al. 2009) was used to generate the circular genome map. The sequencing depth and coverage map for *S. lateralis* (Figure S1) were assessed by the Python script described in Ni et al. (2023). We designed the primers F553 and R1405 (5′-TGTA TAATTCTTATAACATGA CAAAAATT-3′ and 5′-TGTTCCAATAT CTTTGTGATTTGT-3′) to amplify the fragment with low depth (at ca. 1000 bp) and then sequenced using Sanger sequencing to verify the assembled result (Figure S2). Then, we manually checked the sequences of tRNAs and rRNAs to precisely confirm their positions by aligning them with homolog sequences from *S. septemlineata* using MEGA 11 software (Tamura et al.

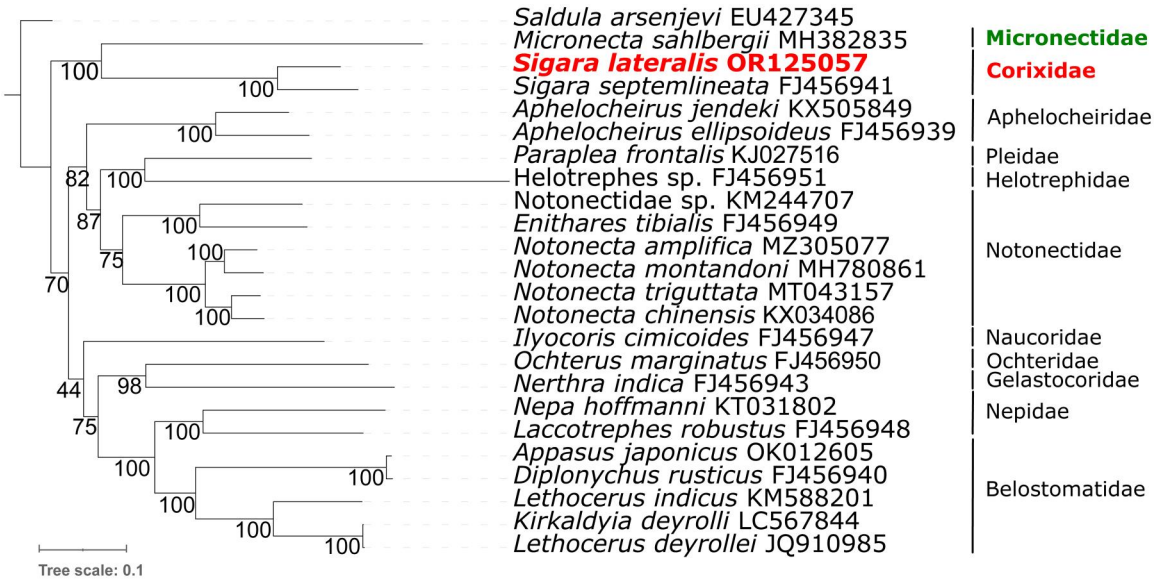

Figure 3. The maximum-likelihood tree of 23 Nepomorpha species. *Saldula arsenjevi* (Leptopodomorpha) was employed as the outgroup. The bootstrap supports are shown at nodes. The mitochondrial genomes of following species were used: *S. arsenjevi* EU427345 (Hua et al. 2008), *A. jendeki* KX505849 (unpublished), *A. ellipsoideus* FJ456939 (Hua et al. 2009), *M. sahlbergii* MH382835 (Zhang et al. 2018), *S. septemlineata* FJ456941 (Hua et al. 2009), *P. frontalis* KJ027516 (Li et al. 2014), *Helotrephes* sp. FJ456951 (Hua et al. 2009), *Notonectidae* sp. KM244707 (Tang et al. 2014), *E. tibialis* FJ456949 (Hua et al. 2009), *N. triguttata* MT043157 (Wang et al. 2023), *N. chinensis* KX034086 (Li et al. 2017), *N. montandoni* MH780861 (Li et al. 2019), *N. amplifica* MZ305077 (Li et al. 2022), *I. cimicoides* FJ456947.1 (Hua et al. 2009), *O. marginatus* FJ456950.1 (Hua et al. 2009), *N. indica* FJ456943.1 (Hua et al. 2009), *N. hoffmanni* KT031802.1 (Zhang et al. 2016), *L. robustus* FJ456948.1 (Hua et al. 2009), *A. japonicus* OK012605 (Han et al. 2022), *L. indicus* KM588201 (Devi et al. 2016), *L. deyrollei* JQ910985 (Li et al. 2017), *K. deyrolli* LC567844 (Nakasako et al. 2020), and *D. rusticus* FJ456940 (Hua et al. 2009).

2021). The location of the control region was determined by the locations of neighboring genes (*s-rRNA* and *trnI*) and aligning with control regions from closely related species. The annotated mitogenome had been deposited in NCBI GenBank under accession no. OR125057. The saturation of Nepomorpha sequences was calculated by using the DAMBE 7.0.35 (Xia 2018). We reconstructed phylogenetic trees of infraorder Nepomorpha comprising all Nepomorpha species with mitochondrial genome released in GenBank, using the maximum-likelihood (ML) and the neighbor-joining (NJ) methods. *Saldula arsenjevi* (EU427345) (Hua et al. 2008) was employed as the outgroup because it belongs to the infraorder Leptopodomorpha, which was the sister group of the infraorder Nepomorpha (Wheeler et al. 1993). Nucleotide sequences of all mitochondrial PCGs from each species were retrieved according to GenBank annotations and then aligned using MAFFT (Katoh et al. 2002). The ML analysis was conducted using IQtree2 software with the best-fitting model GTR + F + I + G4 and 1000 bootstrap replicates to assess the node support (Minh et al. 2020); the NJ analysis was conducted with 1000 bootstrap replicates using MEGA 11 software (Tamura et al. 2021).

Results

Mitogenome organization

The completely circular mitogenome of *S. lateralis* is 15,725 bp long, including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and one AT-rich region (Figure 2). Nine PCGs (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad6*, and *cytb*) and 14 tRNAs (*trnI*, *trnM*, *trnW*, $trnL^{(taa)}$, *trnK*, *trnD*, *trnG*, *trnA*, *trnR*, *trnN*, $trnS^{(gct)}$, *trnE*, *trnT*, and $trnS^{(tga)}$) are encoded in the major strand (J strand), while four PCGs (*nad5*, *nad4*, *nad4l*, and *nad1*) and the other eight tRNA genes (*trnQ*, *trnC*, *trnY*, *trnF*, *trnH*, *trnP*, $trnL^{(tag)}$, and *trnV*) are on the minor strand (N strand). The small and large subunits of rRNA (*s-rRNA* and *l-rRNA*) are 763 bp and 1253 bp in length, respectively. The *s-rRNA* is located between *trnV* and the AT-rich region, while the *l-rRNA* is located between $trnL^{(tag)}$ and *trnV*. The AT-rich region in the mitochondrial genome of *S. lateralis* was located between *s-rRNA* and *trnI* with a length of 1210 bp. The total nucleotide composition of *S. lateralis* mitogenome is A (41.84%), T (34.12%), G (10.35%), and C (13.69%), with an overall A + T content (75.96%).

Phylogenetic analysis

The saturation tests showed that the overall observed index of substitution saturation (Iss) (0.3268) is significantly lower than the critical Iss value (Iss. c) (Iss. c = 0.8461, $p < .05$) (Table S1). Twelve PCGs, except *atp8*, all have significantly lower Iss than their Iss. c, which indicated that the Nepomorpha sequences in this study have not been saturated. Based on 13 PCGs, the ML tree showed that the Corixidae and Micronectidae cluster to a clade of Corixoidea with 100% bootstrap supports (Figure 3). *S. lateralis* is strongly supported as a sister to *S. septemlineata* with bootstrap support values of 100%.

Discussion and conclusions

The complete mitochondrial genome of *S. lateralis* has a length of 15,725 bp and is biased toward A + T. The

phylogenetic analyses based on the mitogenome of 23 Nepomorpha species supported *S. lateralis* closely related to *S. septemlineata*. Micronectidae and Corixidae are grouped as Corixoidea in Nepomorpha, which was also recovered by Zhang et al. (2018). The Corixidae contains many species, but only two of the mitochondrial genomes of species are in the family Corixidae on NCBI, so we had to reconstruct phylogenetic trees of the infraorder Nepomorpha. Limited taxonomic coverage caused the phylogenetic relationships among the Corixidae species not to be robust (Chen et al. 2024); future studies should acquire more genetic information on Corixid species to infer their phylogenetic relationships accurately. The complete mitogenome of *S. lateralis* first reported in the present study will contribute to further studies on the taxonomy and systematics of Corixidae, and the result of phylogenetic trees provides reliable molecular data on the phylogeny of the family Corixidae.

Ethical approval

The material involved in the article does not involve ethical conflicts. This species is neither endangered on the CITES catalogue nor collected from a natural reserve, so it did not need specific permissions or licenses. All collection and sequencing work were strictly executed under local legislation and related laboratory regulations to protect wild resources.

Author contributions

KW: conceptualization, methodology, formal analysis, and writing – original draft. JG: conceptualization, writing – review and editing. XL and ML: conceptualization, sample collection, and modified the article. CD: study design, funding acquisition, and writing – review and editing. YL: conceptualization, funding acquisition, project administration, and writing – review and editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are openly available in the NCBI GenBank database at https://www.ncbi.nlm.nih.gov/genbank/, accession number OR125057. The associated BioProject, Bio-Sample, and SRA numbers are PRJNA978975, SAMN35563314, and SRR24800582, respectively.

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