MITOCHONDRIAL DNA PART B: RESOURCES
2024, VOL. 9, NO. 8, 1020–1023
https://doi.org/10.1080/23802359.2024.2387261

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MITOGENOME REPORT

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The complete mitochondrial genome of *Heptathela kimurai* (Araneae: Heptathelidae)

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ABSTRACT

Heptathela kimurai (Kishida, 1920) is a spider that belongs to the family Heptathelidae which is a basal lineage of spiders. The molecular information of ancestral species belonging to families like Heptathelidae is comparatively limited when compared to spider species from derived families. Here we present the complete mitochondrial genome sequence (mtDNA) of *H. kimurai*. The sequence was obtained using massively parallel sequencing technology. The circular genome was 14,224 bp in length, and the AT content was 69.53%. The *H. kimurai* mitochondrial genome contains 13 protein-coding genes (PCGs), 21 tRNA genes, and 2 rRNA genes. The majority of PCGs were found in the heavy strand.

ARTICLE HISTORY
Received 29 April 2024
Accepted 26 July 2024

KEYWORDS
Kimura-gumo(japanese); basal lineage of spider; phylogenetic tree; long-read sequencing

Introduction

Heptathela kimurai (Kishida, 1920) belongs to the family Heptathelidae, the most basal lineage of extant spiders. This spider retains ancestral traits such as a segmented abdomen and complex spinning organs. Described in 1920, *H. kimurai* is classified under the sub-order Mesothelae and has a body length of approximately 1.5 cm (Figure 1). This spider: *H. kimurai* is found primarily in Japan and other parts of Asia (Tanikawa 2015). Among the more than 52,000 spider species reported to date (World Spider Catalog 2024: https://wsc.nmbe.ch), molecular information of ancestral spiders is essential for understanding phylogenetic relationships. Among the spiders belonging to Heptathelidae, *Songthela hangzhouensis* (Chen et al. 1981) is the only species for which the mitochondrial genome has been determined (Qiu et al. 2005). Moreover, as *H. kimurai* possesses ancestral traits, it plays a crucial role in comprehending the evolution of spider silk. In recent years, molecular phylogenetic analyses of silk materials have become increasingly prevalent, revealing an understanding of their highly diverse evolution (Kono et al. 2020; Kono et al. 2021; Arakawa et al. 2021). Studying this species provides valuable insights into how spiders, which utilize a variety of silks, have acquired such diverse silk-producing abilities. The establishment of molecular information on *H. kimurai* serves as a stepping stone towards unraveling the origins and evolutionary history of spiders and their remarkable silk-spinning capabilities.

Materials and methods

The sample of *H. kimurai* was collected from Kagoshima, Japan (31°32′34.0″N 130°28′24.8″E) on 1 September 2021. One specimen was used in the subsequent process and this sample was identified by DNA barcoding of COI region. The specimen was preserved at −80 °C in Institute for Advanced Biosciences, Keio University (https://www.iab.keio.ac.jp, contact person: Nobuaki Kono, nkono.a3@keio.jp) with the voucher ID7773. The genomic DNA extraction and genome sequencing were conducted according to previous studies (Arakawa et al. 2018; Kono et al. 2021) and manufacturer's

Figure 1. *Heptathela kimurai*, female (Akio Tanikawa).

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2024.2387261.

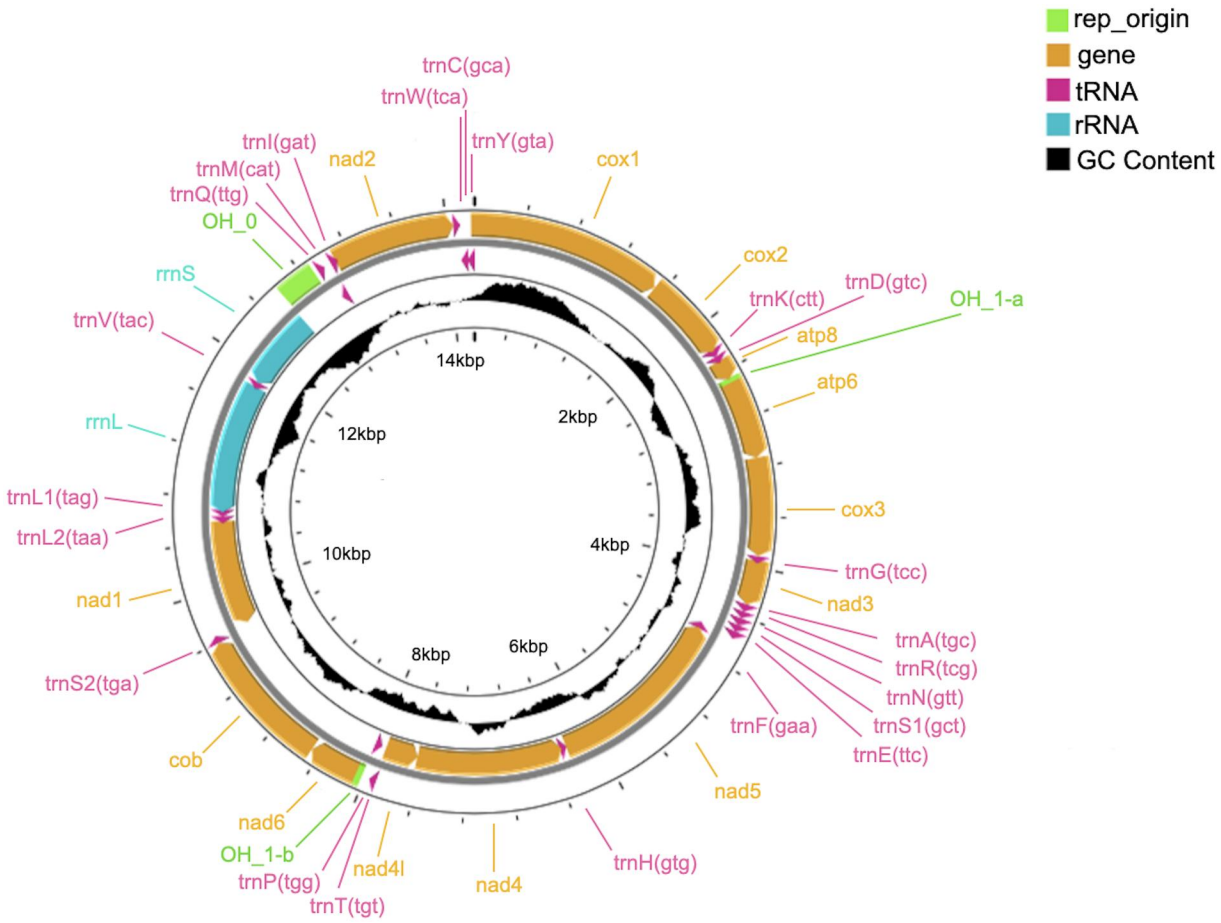

Figure 2. Mitochondrial genome maps for *Heptathela kimurai* (LC807691).

protocols. The genomic DNA was extracted from the whole body. The prepared sequence library was sequenced using the Illumina NovaSeq 6000 platform with 150-bp paired-end reads. Sequenced reads were submitted to the DNA Data Bank of Japan (DDBJ), a member of the International Nucleotide Sequence Database Collaboration (INSDC), with DRA017660 in PRJDB17283. The full-length read of the mitochondrial genome was assembled using NOVOPlasty 4.3.1(Dierckxsens et al. 2017). Mitochondrial annotation was performed using the MITOS Web Server (Bernt et al. 2013). Genes were also manually curated based on closely related species mitochondrial genome sequences. We made the Genome Circular Map by Proksee (Grant et al. 2023) and calculated the reads coverage of the mitochondrial region by SAMtools depth (Li et al. 2009). A phylogenetic tree was created based on Maximum Likelihood (ML) using 16 spider mitogenome sequences. Sequence information for each of the species used to create the phylogenetic tree is shown in Table S1. The dataset was made up of 13 PCGs. These amino acid sequences were obtained from NCBI (Schoch et al. 2020) and aligned with MAFFT Online version (Katoh et al. 2019), followed by phylogenetic analysis with RAxML version 8.2.13 (Stamatakis 2014). *Centruroides vittatus* (Scorpion) was used as an outgroup. The phylogenetic tree was visualized using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The complete mitochondrial genome of *H. kimurai* has been submitted to DDBJ under accession number (LC807691). The complete mitochondrial genome was 14,224 bp in length with AT/GC contents of 69.53 and 30.47% ($A = 35.61\%$, $T = 33.92\%$, $G = 10.22\%$, $C = 20.25\%$), respectively. The coverage depth of the whole genome sequence is shown in Figure S1. Mitochondrial genome annotation represented 13 protein-coding genes (cox1, cox2, cox3, atp6, atp8, nad1, nad2, nad3, nad4-0, nad4l, nad5, nad6, and cob), 21 tRNA genes, and two rRNA genes (Figure 2). Four protein-coding genes (nad1, nad4l, nad4-0, and nad5) were encoded in the light strand and the remaining nine genes were encoded in the heavy strand. Both rRNAs were encoded on the light strand and located between tRNA(Ile) and tRNA(Leu) and separated by the tRNA(Val)gene. The phylogenetic tree created using 13 protein-coding genes from 16 spider species and *C. vittatus* (Scorpion) is shown in Figure 3.

Discussion and conclusion

In this study, the complete mitochondrial genome sequence of *H. kimurai* is reported for the first time. The size of the mitochondrial genome is similar to other spiders, but it has 21 tRNA genes, one less than other derived spiders, which have 22 tRNA genes. This is the same number as *C. vittatus*,

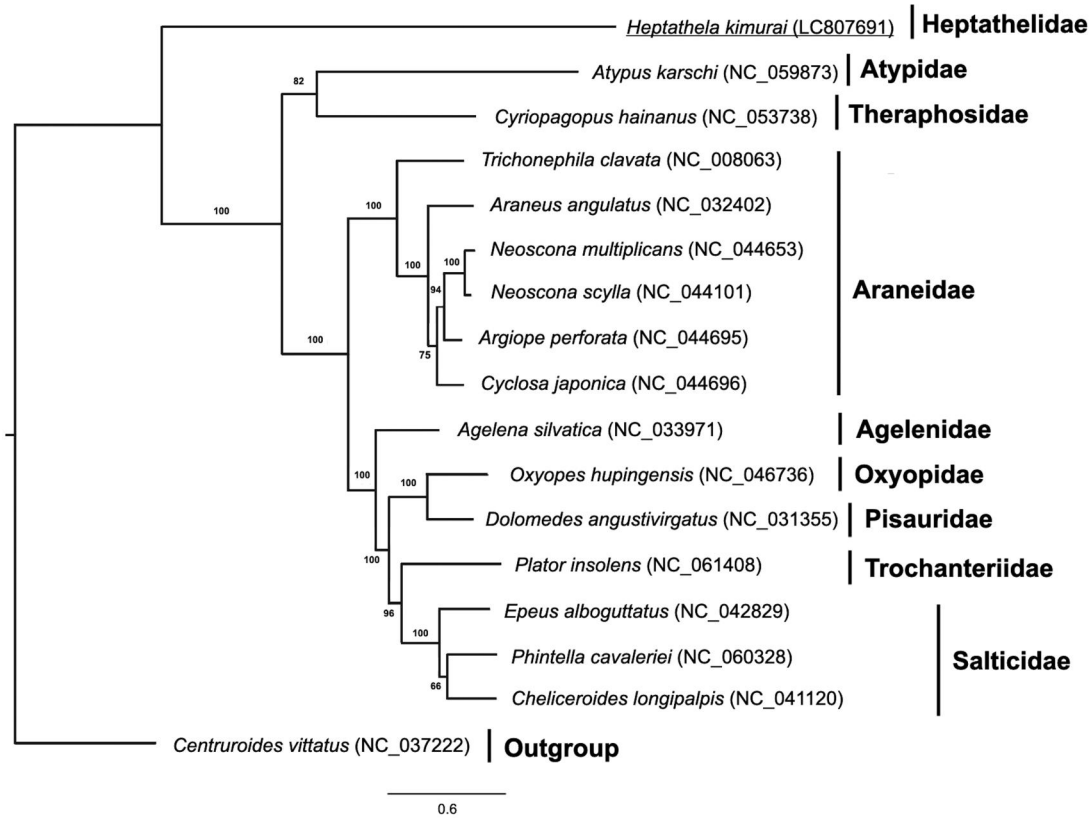

Figure 3. Phylogenetic tree showing the relationship between *H. kimurai* (LC807691) and 15 other spiders. The following sequences were used: *A. karschi* (NC_059873.1), *C. hainanus* (NC_053738.1: Chen et al. 2020), *T. clavata* (NC_008063.1), *A. angulatus* (NC_032402.1), *N. multiplicans* (NC_044653.1: Xu et al. 2018), *N. scylla* (NC_044101.1: Xu et al. 2019), *A. perforata* (NC_044695.1: Yang et al. 2019), *C. japonica* (NC_044696.1), *A. silvatica* (NC_033971.1), *O. hupingensis* (NC_046736.1: Yang et al. 2019), *D. angustivirgatus* (NC_031355.1), *P. insolens* (NC_061408.1), *E. alboguttatus* (NC_042829.1: Yang et al. 2018), *P. cavaleriei* (NC_060328.1), and *C. longipalpis* (NC_041120.1: Chen et al. 2018).

which is located in the spider's ancestral lineage (Table S1). The gene order in the mitochondrial genome of *H. kimurai* was largely similar to that found in previously determined spider mitogenomes (Wang et al. 2016). The phylogenetic tree creation with other spider species and *C. vittatus* using the 13 protein-coding genes shows the validity of assembly results. Previous studies have shown that among the spiders used in this phylogenetic tree, those belonging to Heptathela are the most closely related ancestral spiders to scorpions (Zhu et al. 2019). Molecular information on ancestral spider species has been insufficient. However, it is believed that the molecular information on *H. kimurai* will significantly contribute to the advancement of arachnology. This is because the development of molecular information on ancestral spiders will lead to significant advances in the macro-phylogenetic analysis of spiders and related research. Furthermore, since the ancestral spiders, unlike the derived spiders, do not have an aerial web, the development of molecular information on the ancestral spiders and comparison with the derived spiders will help to elucidate the origin of the spider silk.

Acknowledgments

The authors thank Maaya Domon for technical support with sequencing.

Ethical approval

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

Authors' contributions

This research project was designed by NK and developed by CI and NK. The sample was collected by HN and AT. Data analysis and initial manuscript drafting were completed by CI and PY. All authors contributed to manuscript edits and finalization, approved the final version of the manuscript for publication.

Disclosure statement

No potential conflict of interest was reported by the authors. HN is an employee of Spiber Inc., however, Spiber Inc. had no role in study design, data analysis, and data interpretation.

Funding

This study was supported by the Japan Society for the Promotion of Science under Grant number 21H02210/Grant-in-Aid for Scientific Research (B), 24K21257/Grant-in-Aid for Challenging Research (Pioneering), and Grant number 16H06279 (PAGS)/Platform for Advanced Genome Science, Taikichiro Mori Memorial Research Grant, Yamagata Prefecture, Tsuruoka City, and Keio University Academic Development Funds.

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Data availability statement

The data that support the findings of this study are openly available in DDBJ at (https://www.ddbj.nig.ac.jp), accession number of (LC807691).

The associated BioProject, SRA, and BioSample numbers are PRJDB17283, DRA017660, and SAMD00728320, respectively.

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