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A mitogenomic phylogeny of spiders and complete mitochondrial genome of *Cyriopagopus hainanus* (Araneae:Theraphosidae)

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ABSTRACT

We describe the complete mitochondrial genome sequence of *Cyriopagopus hainanus*, a spider in the family of Theraphosidae and endemic to Hainan Island, China. Phylogenetic analyses using mitogenomes of 32 spider species from 20 families strongly supported our sample is sister to *Cyriopagopus schmidti*. This is also the largest mitogenomic phylogeny of spiders to date. The mitogenomic length of *C. hainanus* is 13,874 bp, including 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and 1 control region. The complete mitochondrial genome of *C. hainanus* will contribute to studies of mitogenomic evolution and trait evolution in spiders.

ARTICLE HISTORY

Received 3 January 2020 Accepted 7 January 2020

KEYWORDS

Mitogenome; *Cyriopagopus hainanus*; spider; mitogenomic phylogeny; Hainan Island

Spiders (Order Araneae) are a hyperdiverse arthropod group comprising >48,000 described species distributed in \sim 120 families (World Spider Catalog 2019). Spiders in the family Theraphosidae are often large and hairy species found in tropical and subtropical regions around the world, with most living in a ground burrow (Zhu and Zhang 2008). Among them, *Cyriopagopus hainanus* is only distributed in Hainan Island, China (Liang et al. 1999). In this study, we present the largest mitogenomic phylogeny of spiders so far and the complete mitochondrial genome of *C. hainanus*, which would contribute to studies of mitogenomic evolution and trait evolution in spiders.

Total genomic DNA was extracted from the legs of one female *Cyriopagopus hainanus* collected on 23 October 2019 in the Licai Farm, Tianya District, Sanya City, Hainan Island, China (location: N18°28′, E109°17′; voucher number: BL-HXW-001, stored in Hainan Academy of Forestry). A genomic library with an average insert size of approximately 500 bp was constructed by NEB Next® UltraTM DNA Library Prep Kit for Illumina® and sequenced as 150 bp paired-end on an Illumina HiSeq X Ten platform in Sangon Biotech (Shanghai) Co., Ltd. The generated reads were filtered, trimmed, and mapped to a reference mitogenome of *Cyriopagopus schmidti* (GenBank: NC_005925) and then assembled using Geneious Prime® 2019.2.1.

To explore the phylogenetic position of *C. hainanus* on a spider tree, mitogenomes of 31 spider species were downloaded from Genebank. After extracting the 13 proteincoding genes and the 2 ribosomal RNA genes from each

genome, we aligned individual genes using Mafft (Katoh et al. 2009) and combined them into a concatenated dataset. We conducted phylogenetic analyses in RAxML v8.2.10 (Stamatakis 2014) using the GTRGAMMA model and 200 ultrafast bootstraps (-f a). The partitioned (by locus) Maximum Likelihood (ML) tree highly supported (100%) the phylogenetic position of our sample as sister to C. schmidti (Figure 1) that distributed in Northern Vietnam and Guangxi Province, China (Wirth 1991; Wang et al. 1993). These two species are 93% identical in mitogenome sequences. Their geographic distribution pattern can also be found in other animal groups around this area (e.g. Liang et al. 2018), which implies that the endemic species in Hainan Island may have a continental origin and are closely related to Northern Vietnam and Guangxi Province, China. Our concatenated analyses of the mitogenomic data resulted in a completely resolved tree with 100% bootstrap support for almost all major clades. The phylogenetic topology is almost identical to those reconstructed by transcriptomic data (Garrison et al. 2016; Fernández et al. 2018) and several mitochondrial and nuclear gene markers (Wheeler et al. 2017).

The complete mitogenome of *C. hainanus* is circular and 13,874 bp in length. It contains 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and a putative control region (GenBank accession number: MN877932) with annotation in MITOS Web Server (Bernt et al. 2013). It has the same gene order with other species in Mygalomorphae and the full mtDNA sequence contains 30.4% of GC.

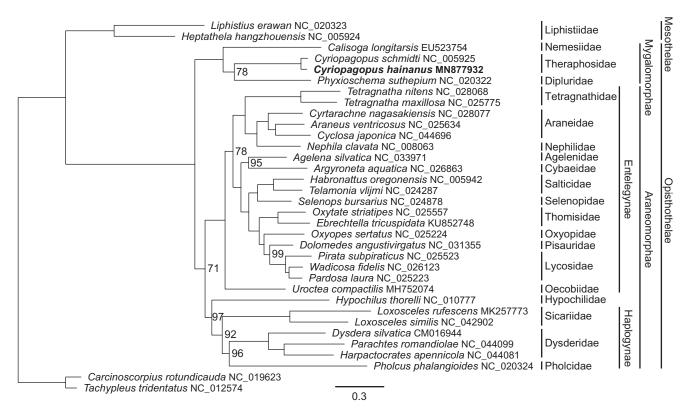


Figure 1. Mitogenomic phylogeny of Araneae, comprising 32 spider taxa from 20 families, was constructed using 13 mitochondrial protein-coding genes and the 2 ribosomal RNA genes by concatenated analysis with Maximum Likelihood (ML) method. Two horseshoe crabs, Carcinoscorpius rotundicauda and Tachypleus tridentatus were used as outgroups. Bootstrap support at major nodes is 100% unless indicated on the tree. GenBank accession numbers of all species used in this study are also shown by the species name.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This study was supported by Hainan Provincial Natural Science Foundation of China [No. 317200 to Haixia Wu] and the National Natural Science Foundation of China [No. 31301894 to Bin Liang].

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