

Characterization of the complete mitogenome sequence of the giant panda tick *Haemaphysalis hystricis*

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ABSTRACT

The blood-sucking tick *Haemaphysalis hystricis* is a common ectoparasite of the giant panda and represents a significant threat to both wild and captive populations. Herein, the complete mitogenome of *H. hystricis* was sequenced using Illumina sequencing technology. The complete mitogenome sequence was 14,715 bp in size and encoded 37 genes including 13 protein-coding genes, 22 transfer RNAs, and two ribosomal RNAs. Phylogeny revealed that two isolates of *H. hystricis*, regardless of host origins and locations, grouped together and had a closer relationship with *Haemaphysalis longicornis* than other tick species among the genus *Haemaphysalis*. The cumulative mitochondrial DNA data provides novel resources for genetic and phylogenetic studies of *Haemaphysalis* ticks.

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The giant panda, *Ailuropoda melanoleuca*, is regarded as the flagship species for wildlife conservation in China (O'Brien et al. 1994; Wang et al. 2018). The blood-sucking ticks are common ectoparasites found in giant pandas and can cause dermatitis, anemia, and even death in highly infested wild and captive populations (Qiu and Mainka 1993; Cheng et al. 2013; Wang et al. 2018). About 13 tick species belonging to three genera, namely *Haemaphysalis*, *Ixodes* and *Dermacentor*, have been identified from the giant panda based on morphological studies so far (Wang et al. 2018). However, current knowledge progress linked these ticks is limited in their morphology and biology, there are still major gaps in the understanding of the ectoparasites at the molecular level, especially in genetics and molecular epidemiology owing to lacking suitable genetic markers (Cheng et al. 2013; Wang et al. 2018). Mitochondrial DNA has proven to be a valuable source of molecular markers and is being widely applied for genetics and molecular identification of many zoonotic ectoparasites including ticks (Hwang et al. 2001; Cheng et al. 2013; Liu et al. 2013; Burger et al. 2014). In this study, we determined the complete mitogenome sequence of a tick representative *Haemaphysalis hystricis* from the giant panda in Sichuan Province of China and aimed to provide novel mitochondrial resources to this ectoparasite.

The tick samples ($n=2$) were collected from a naturally infected adult male giant panda housed in the Dujiangyan Base of the China Conservation and Research Center for the

Giant Panda, Sichuan Province of Southwest China (30°59'N, 103°37'E). Two ticks were identified as *H. hystricis* females according to the morphological key of Tanskul and Inlao (1989) and the molecular sequencing of the mitochondrial 16S ribosomal DNA gene (Takano et al. 2014). One tick specimen was used for DNA extraction and another was archived in the Parasitological Museum of Sichuan Agricultural University (Sichuan, China) under collection numbers XY2018_14. The mitogenome was sequenced using the Illumina HiSeq platform (Novogene, Tianjin, China), and the genome assembly and annotation were performed as previously described (Xie et al. 2019). The complete sequence has been deposited in GenBank under accession number: MT013253.

The mitogenome sequence of *H. hystricis* was 14,715 bp in size with 77.3% AT and encoded 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes. Among the 37 genes, seven PCGs and 14 tRNAs were located on the forward strand (H-strand), whereas the remaining genes were transcribed on the reverse strand (L-strand). Thirteen PCGs, except for *nad4* and *nad6* deduced to use an incomplete stop codon 'T', were predicted to use the typical TAA or TAG as the stop codons. Twenty-two tRNA genes ranged from 51 bp (tRNA^(AGA)-Ser) to 68 bp (tRNA-Lys) in size and had a typical clover-leaf like secondary structure. The sizes of two rRNA genes were 696 bp (12S) and 1204 bp (16S), respectively, and present between tRNA-Leu and tRNA-Ile with a

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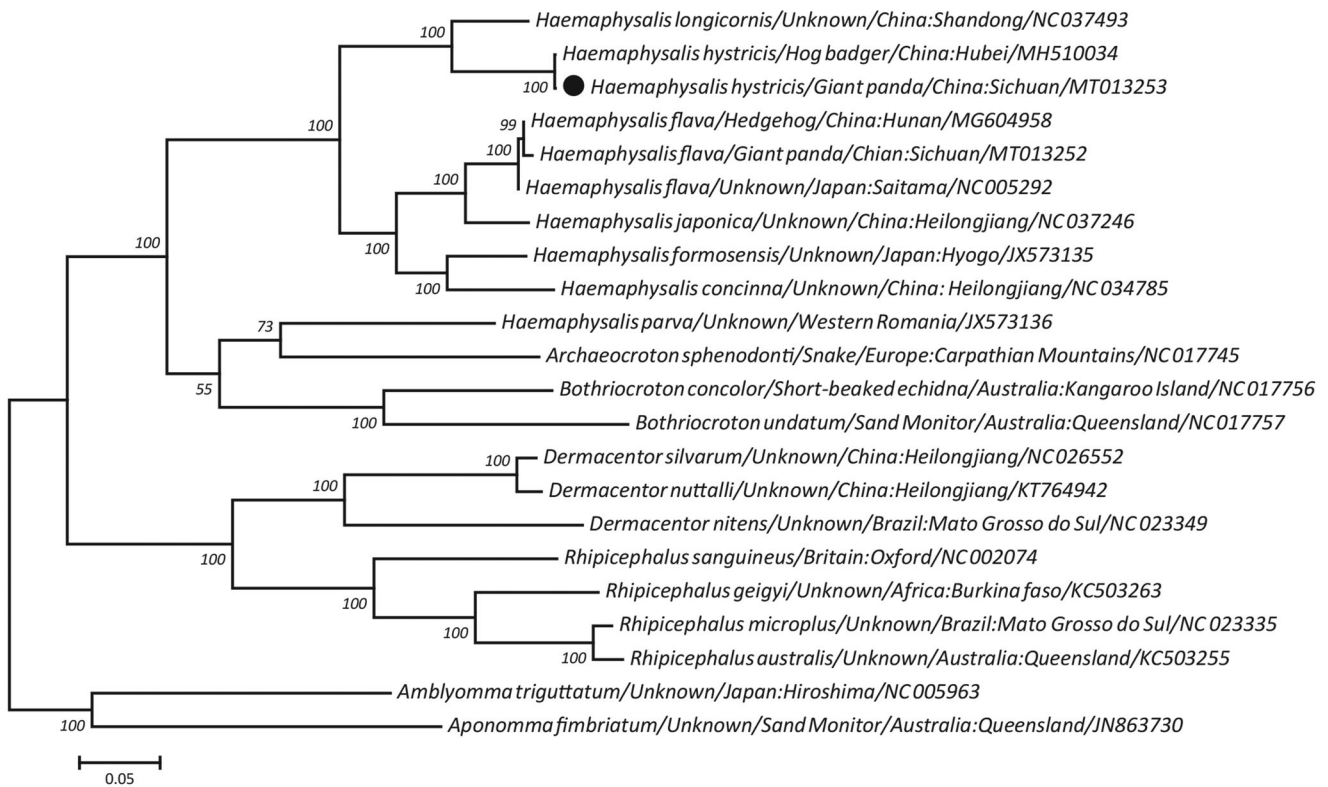


Figure 1. Maximum-likelihood tree inferred from concatenated amino-acid sequences of twenty mitochondrial PCGs of *H. hystricis* and other related ticks, utilizing MtArt + G model and 100,000 bootstrap replications. Both *Amblyomma triguttatum* and *Aponomma fimbriatum* were used as the outgroups. Values lower than 50% are not shown. The black cycle represents the species in this study.

separation by tRNA-Val. The control region (also known as D-loop region) was located between tRNA-Leu and tRNA-Cys, similar to other tick species, suggesting its conservation and function in regulation of transcription and control of DNA replication (Clayton 1991).

Building on a concatenated amino acid dataset of 12 protein-coding genes from *H. hystricis* and 20 other ticks, the maximum-likelihood (ML)-based phylogeny demonstrated that two isolates of *H. hystricis*, regardless of host origins and locations, clustered together and were more closely related to *Haemaphysalis longicornis* than to other ticks in the genus *Haemaphysalis*, with 100% bootstrap confidence (Figure 1), supporting their species validity among the family Ixodidae. In addition, the sister genera including *Archaeocroton*, *Bothriocroton*, *Dermacentor*, and *Rhipicephalus* in this topology were treated as monophyletic relationships with *Haemaphysalis* in Ixodidae, consistent with recent molecular studies (Burger et al. 2013; Geng et al. 2017; Tian et al. 2019). Taken together, the *H. hystricis* mitogenome data sequenced here provides a novel data resource for genetic and evolutionary biological studies of *Haemaphysalis* ticks.

Disclosure statement

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

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