

The mitochondrial genome of the giant panda tick *Haemaphysalis flava* (Acari, Ixodidae) from Southwest China

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ABSTRACT

The tick *Haemaphysalis flava* (Acari, Ixodidae) is an obligatory blood-feeding ectoparasite of the giant panda and is also a vector for transmission of pathogenic microorganisms. In this study, the complete mitochondrial genome of this tick was sequenced through Illumina sequencing technology. The genome was 14,699 bp in length and encoded 37 genes including 13 protein-coding genes, 22 transfer RNAs and two ribosomal RNAs. Phylogeny revealed that three isolates of *H. flava*, regardless of host origins and locations, clustered together and formed a monophyletic relationship with *Haemaphysalis japonica*, supporting their species validity among the genus *Haemaphysalis*. These cumulative mitochondrial DNA data provides insights into phylogenetic studies among *Haemaphysalis* ticks.

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The giant panda (*Ailuropoda melanoleuca*), one of the world's most endangered animals, is regarded as a flagship species for wildlife conservation in China (O'Brien et al. 1994; Peng et al. 2001). Among ectoparasites of the giant panda, ticks represent a significant threat to both wild and captive populations. These blood-feeding parasites attach to the skin of giant pandas and cause dermatitis, anemia, weight loss and even death in highly infested animals (Cheng et al. 2013; Wang et al. 2018). Also, they are vectors and can transmit pathogenic bacteria and viruses, leading to secondary infections (Zhang et al. 2010). Until now there have been more than 13 tick species identified from giant pandas based on morphological characteristics (Cheng et al. 2013; Wang et al. 2018). Although morphological identification is convenient and economical, it is labor-consuming, time-costing and often inaccurate for close-related tick species, especially those infesting wild animals (Qin et al. 2011). Molecular approach, based on mitochondrial DNA (mtDNA), is proven to be a valuable complementary tool for overcoming this limitation and has been widely used for identification and characterization of many ectoparasites including ticks (Hwang et al. 2001; Cheng et al. 2013; Liu et al. 2013; Burger et al. 2014). Here, we reported the complete mitochondrial genome sequence of a tick representative *Haemaphysalis flava* (Acari, Ixodidae) from the giant panda in Sichuan Province, China and added novel mtDNA data to this ectoparasite.

The parasite samples were obtained from a naturally infected adult female giant panda housed in the China

Conservation and Research Center for the Giant Panda at Ya'an (30°04'N, 102°59'E), Sichuan Province of Southwest China. After morphological identification, all ticks ($n = 3$) were identified as *H. flava* females according to the taxonomic key of Cheng et al. (2013) and the molecular sequencing by amplification of the second internal transcribed spacers (ITS2) of nuclear ribosomal DNA (Chitimia et al. 2009). One tick specimen was used for DNA extraction, and the remaining two were fixed in 5% formalin solution and archived in the Parasitological Museum of Sichuan Agricultural University (Sichuan, China) under collection numbers XY2018_12-13. The mtDNA was sequenced using the Illumina HiSeq platform (Novogene, Tianjin, China). The genome was assembled using MITObim (Hahn et al. 2013) and gene annotation was achieved by MITOS (Bernt et al. 2013).

The complete mitochondrial genome of *H. flava* was 14,699 bp in length (GenBank accession no. MT013252) with 77.0% AT and encoded 13 protein-coding genes, 22 tRNA genes and two rRNA genes. Nine PCGs and 20 tRNAs were located on the forward strand (H-strand) while the remaining genes were transcribed on the reverse strand (L-strand). Among the 13 protein-coding genes, except for *cox3*, *nad5* and *nad4* deduced to use an incomplete stop codon 'T', the rest were predicted to use the typical TAA or TAG as the stop codons. Twenty-two tRNA genes ranged from 55 bp (tRNA^(AGA)-Ser) to 67 bp (tRNA-Gln) in length and all had a typical clover-leaf like secondary structure, except for tRNA-Cys and tRNA^(GCT)-Ser. Both 12S and 16S rRNAs were

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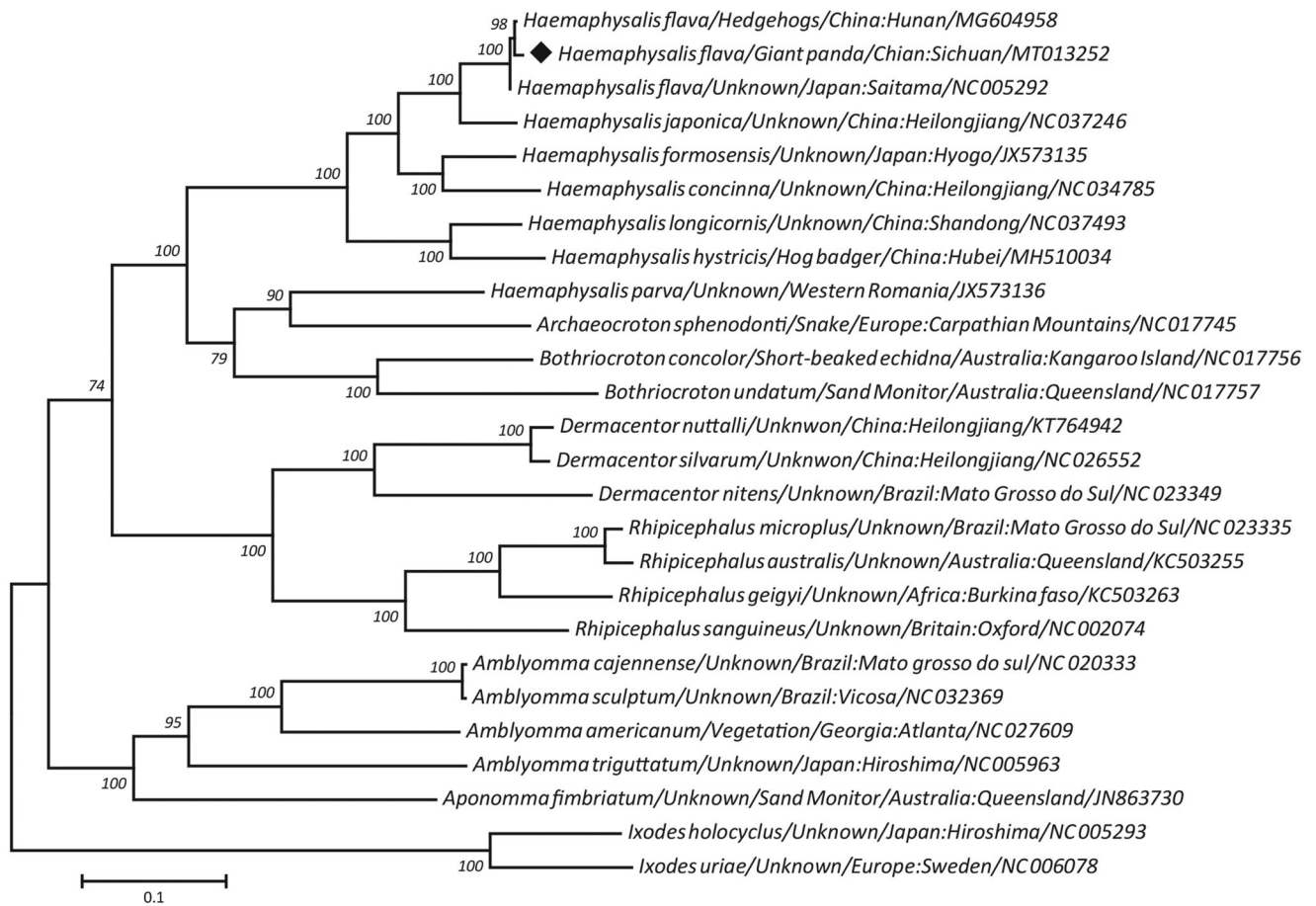


Figure 1. Maximum likelihood tree inferred from concatenated amino-acid sequences of twenty mt protein-coding genes of *H. flava* and other related ticks, utilizing MtArt model and after 1,000,000 bootstrap replications (<50% support not shown). The black diamond sign represents the species in this study.

700 bp and 1200 bp in length, respectively, and placed between tRNA-Leu and tRNA-Ile with a separation by tRNA-Val. Two non-coding regions were placed between 12S and tRNA-Ile and between tRNA-Leu and tRNA-Cys, respectively, similar to other tick species, suggesting their conservation and function in regulation of transcription and control of DNA replication (Clayton 1991).

A maximum-likelihood (ML) phylogeny was reconstructed on a concatenated amino acid dataset of 12 protein-coding genes from *H. flava* and 23 other ticks, using species of *Ixodes* as outgroups. As shown in Figure 1, this phylogenetic tree demonstrates three isolates of *H. flava*, regardless of host origins and locations, clustered together and formed a monophyletic relationship with *Haemaphysalis japonica* in the genus *Haemaphysalis*, with 100% bootstrap confidence, supporting their species validity among the family Ixodidae. In addition, within this topology, each sister genus *Amblyomma*, *Bothriocroton*, *Dermacentor*, *Rhipicephalus* or *Aponomma* was also treated as a monophyletic group in Ixodidae, consistent with recent molecular studies (Burger et al. 2013; Geng et al. 2017; Tian et al. 2019). In conclusion, the complete mtDNA of *H. flava* sequenced here provides novel molecular evidence for phylogenetic and taxonomic position of this tick species.

Disclosure statement

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

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References

- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2): 313–319.
- Burger TD, Shao R, Barker SC. 2013. Phylogenetic analysis of the mitochondrial genomes and nuclear rRNA genes of ticks reveals a deep phylogenetic structure within the genus *Haemaphysalis* and further elucidates the polyphyly of the genus *Amblyomma* with respect to *Amblyomma sphenodonti* and *Amblyomma elaphense*. *Ticks Tick-Borne Dis.* 4:265–274.
- Burger TD, Shao R, Barker SC. 2014. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus (Boophilus) microplus*, contains a cryptic species. *Mol Phylogenet Evol.* 76:241–253.
- Cheng WY, Zhao GH, Jia YQ, Bian QQ, Du SZ, Fang YQ, Qi MZ, Yu SK. 2013. Characterization of *Haemaphysalis flava* (Acari: Ixodidae) from Qingling subspecies of giant panda (*Ailuropoda melanoleuca qinlingensis*) in Qinling mountains (Central China) by morphology and molecular markers. *PLoS One.* 8(7):e69793.
- Chitimia L, Lin RQ, Cosoroaba I, Braila P, Song HQ, Zhu XQ. 2009. Molecular characterization of hard ticks from *Romania* by sequences

- of the internal transcribed spacers of ribosomal DNA. *Parasitol Res.* 105(5):1479–1482.
- Clayton DA. 1991. Replication and transcription of vertebrate mitochondrial DNA. *Annu Rev Cell Biol.* 7(1):453–478.
- Geng J, Zheng A, Zou Z, Zhang X. 2017. The complete mitochondrial genome and phylogenetic analysis of *Haemaphysalis longicornis* Neumann (Acari: Ixodidae). *Mitochondrial DNA B Resour.* 2(2):856–857.
- Hahn C, Bachmann L, Chevreux B. 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucleic Acids Res.* 41(13): e129–e129.
- Hwang UW, Park CJ, Yong TS, Kim W. 2001. One-step PCR amplification of complete arthropod mitochondrial genomes. *Mol Phylogenet Evol.* 19(3):345–352.
- Liu GH, Chen F, Chen YZ, Song HQ, Lin RQ, Zhou DH, Zhu XQ. 2013. Complete mitochondrial genome sequence data provides genetic evidence that the brown dog tick *Rhipicephalus sanguineus* (Acari: Ixodidae) represents a species complex. *Int J Biol Sci.* 9(4):361–369.
- O'Brien S J, Pan W, Lu Z. 1994. Pandas, people and policy. *Nature.* 369(6477):179–180.
- Peng JJ, Jiang ZG, Hu JC. 2001. Status and conservation of giant panda (*Ailuropoda melanoleuca*): a review. *Folia Zool.* 50:81–88.
- Qin XC, Tian JH, Wang JB, Lu X, Sun QZ, Jin D, Zhou DJ, Xu JG, Zhang YZ. 2011. Identification of *Haemaphysalis longicornis* and *Rhipicephalus microplus*. *Chin J Epidemiol.* 32:608–612.
- Tian J, Ge M, Xu H, Wu T, Yu B, Lei C. 2019. The complete mitochondrial genome and phylogenetic analysis of *Haemaphysalis hystricis* (Parasitiformes: Ixodidae). *Mitochondrial DNA B Resour.* 4(1):1049–1050.
- Wang T, Xie Y, Zheng Y, Wang C, Li D, Koehler A V, Gasser R B. 2018. Parasites of the giant panda: a risk factor in the conservation of a species. *Adv Parasitol.* 99:1–33.
- Zhang H, Wang XH, Fan WF, Yuan M. 2010. Review of parasitosis of giant panda. *Gansu Anim Vet Sci.* 212:40–43.