

Complete mitochondrial genome and phylogenetic analysis of *Ixodes ovatus* (Acari: Ixodidae)

Dandan Jiang^a, Xinyan Lu^b, Chunhong Du^c, Shanbin Hu^d, Zhipang Huang^e and Xing Yang^b

^aSchool of Public Health, Dali University, Dali, P. R. China; ^bIntegrated Laboratory of Pathogenic Biology, College of Preclinical Medicine, Dali University, Dali, P. R. China; ^cYunnan Institute of Endemic Diseases Control and Prevention, Yunnan, P. R. China; ^dAdministration of Yunling Provincial Nature Reserve, Nujiang, Yunnan, PR China; ^eInstitute of Eastern-Himalaya Biodiversity Research, Dali University, Yunnan, China

ABSTRACT

Ixodes ovatus is referred to as an obligatory blood-sucking ectoparasite that is capable to infest both humans and animals. In the present study, the complete mitochondrial genome of *I. ovatus* was sequenced and analyzed using next-generation sequencing (NGS) technology. With a size of 14,520 bp, the entire mitogenome contains 37 genes including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and 3 control regions (D-loops). Based on the 13 PCGs nucleotide sequences, the phylogenetic relationship of *I. ovatus* was analyzed using Maximum-likelihood. As suggested by the results of the obtained phylogeny, *I. ovatus* is most closely associated with *Ixodes hexagonus*. This study is expected to promote further studies on the evolution of Ixodidae.

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Ixodes ovatus (Neumann, 1899) is a species of hard tick with a wide geographical distribution across China, Taiwan, Korea, Japan, Burma, and Thailand (Harry Hoogstraal 1973). With cows, horses, donkeys, deer, and sheep as known hosts, *I. ovatus* can accidentally parasitize humans, thus spreading a wide variety of severe animal and human pathogens, including *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, *Borrelia garinii*, and *Babesia spp.*, etc. (Shimada 2003). Therefore, correct identification of tick species is essential for disease control (Shimada 2003). However, conventional morphological identification requires extensive experience, which may restrict its applications. Since the mitochondrial genome is regarded as an effective genetic marker for species identification, sequencing of their complete mitochondrial genomes is beneficial to the identification and classification of ticks (Taanman 1999).

In this study, *I. ovatus* samples were collected in March 2021 from Tue Village, Nujiang City, Yunnan Province, China (98°48'59.84"E, 26°34'20.56"N) and then preserved in 75% ethanol. The recognition of samples was conducted by Professor Chunhong Du based on the morphological characteristics (Lu et al. 2021). Then, the specimen was deposited in Parasitological Museum, Dali University NO. DLUP2103 (URL: <http://www.dali.edu.cn/jcyxy/xkpt/jcyxsyjsxz/6431.htm>, Contact person: Xing Yang, yang08220013@163.com). The entire DNA was extracted using the standard CTAB technique, for storage at -20 °C before use (Lu et al. 2021). The mitochondrial genome was sequenced on the Illumina NovaSeq platform (Shanghai Personal

Biotechnology Co, Ltd) which was assembled using A5-miseq software (Coil et al. 2015), and genome annotations were performed using the Swiss-Prot web server (<http://www.ebi.ac.uk/uniprot/>).

The mitochondrial genome of *I. ovatus* was determined as 14,520 bp (Genbank accession no. OM317739), involving 13 PCGs (*cytb*, *nad1-6*, *atp8*, *nad4L*, *cox1-3*, and *atp6*), 22 tRNAs, 2 rRNAs. The genetic order of the *I. ovatus* was identical to hard ticks. The entire base composition of the *I. ovatus* mitochondrial genome was determined as 37.54% A, 37.39% T, 16.24% C, 8.83% G. The size of *I. ovatus* small submit rRNA and large submit rRNA was 657 and 1,190 bp, respectively. It was discovered that the length of 22 tRNAs varied from 55 bp (*tRNA-Ser*) to 71 bp (*tRNA-Lys*), with 13 tRNAs encoded on the plus-strand (Thomas et al. 2013).

The GTR + G + I model was applied as the suitable model for sequencing, and the maximum-likelihood method was adopted through the MEGA 7.0 software with 1000 bootstrap replicates (Kumar et al. 2016). As shown in Figure 1, the phylogenetic tree included complete mitogenomes sequences of 28 Ixodidae species previously published on GenBank. As revealed by the phylogenetic analysis, the obtained tree is divided into two phylogroups: Metastricata and Prostricata (Ciloglu et al. 2021). Additionally, it is shown that *I. ovatus* and all the other species within the genus *Ixodes* cluster in a branch with high statistical support, confirming *I. ovatus* within the genus *Ixodes*. The complete mitochondrial genome of *I. ovatus* provides an important molecular resource for

CONTACT Zhipang Huang  huangzp@eastern-himalaya.cn  Institute of Eastern-Himalaya Biodiversity Research, Dali University, Yunnan, China; Xing Yang  yang08220013@163.com  Integrated Laboratory of Pathogenic Biology, College of Preclinical Medicine, Dali University, Dali, P. R. China
Dandan Jiang and Xinyan Lu contributed equally.

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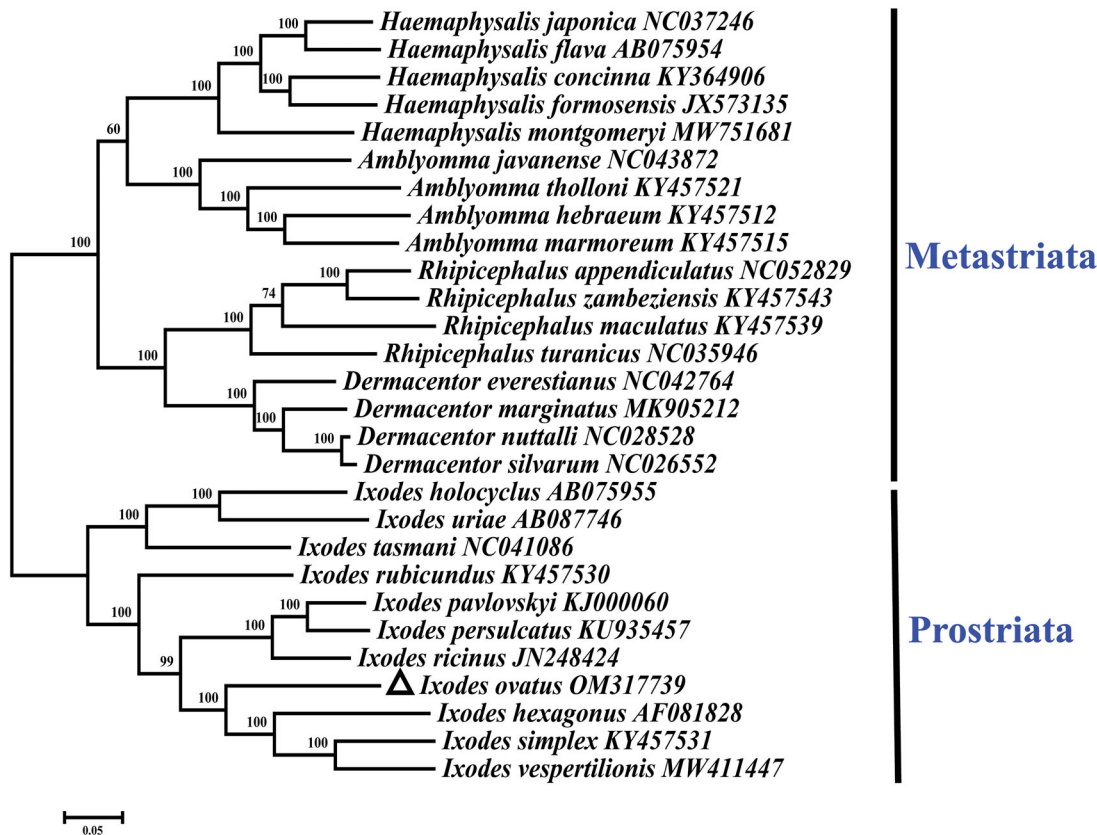


Figure 1. Maximum-likelihood (ML) phylogeny of 28 species of the family Ixodidae based on the 13 concatenated nucleotide sequences of protein-coding genes (PCGs), utilizing the GTR + G + I model and after 1,000 bootstrap replications. The black triangle sign represents the species in this study. Bootstrap support values are shown above the nodes.

further study on the phylogeny of the genus *Ixodes* and of Ixodidae (Kelava et al. 2021).

Ethical approval

This study was approved by the Administration Committee of Experimental Animals, Dali University, Yunnan Province, China.

Author contributions

DDJ conceived the study and wrote the manuscript. XYL carried out the experiments and analyzed the data. CHD, SBH and ZPH contributed to the collection of *I. ovatus* and discussions, XY is responsible for the interpretation of experimental data, critical revision of important knowledge content and final approval of the version to be published.

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 National Center for Biotechnology Information (NCBI) at <https://www.ncbi.nlm.nih.gov>. The accession number of the complete mitochondrial genome is OM317739. The associated BioProject, SRA, and BioSample numbers are PRJNA820144, SRR18494365 and SAMN26982089, respectively.

References

- Ciloglu A, Ibis O, Yildirim A, Aktas M, Duzlu O, Onder Z, Simsek E, Yetismis G, Ellis VA, Inci A. 2021. Complete mitochondrial genome characterization and phylogenetic analyses of the main vector of Crimean-Congo haemorrhagic fever virus: *Hyalomma marginatum Koch*, 1844. *Ticks Tick Borne Dis.* 12(5):101736.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics.* 31(4):587–589.
- Hoogstraal H, Clifford CM, Saito Y, Keirans JE. 1973. *Ixodes (partipaliger) ovatus* Neumann, subgen. *J Med Entomol.* 10(2):157–164.
- Kelava S, Mans BJ, Shao R, Moustafa M, Matsuno K, Takano A, Kawabata H, Sato K, Fujita H, Ze C, et al. 2021. Phylogenies from mitochondrial genomes of 120 species of ticks: Insights into the evolution of the families of ticks and of the genus *Amblyomma*. *Ticks Tick Borne Dis.* 12(1):101577.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 33(7): 1870–1874.
- Lu X, Jiang D, Du C, Yang X. 2021. The complete mitochondrial genome of *Haemaphysalis montgomeryi*. *Mitochondr DNA B Resour.* 6(8):2233–2234.

- Lu X, Zuo X, Jiang D, Yang X. 2021. The complete mitochondrial genome of *Ixodes vespertilionis* (Acari: Ixodidae). *Mitochondr DNA B Resour.* 6(10):3001–3003.
- Shimada Y, Inokuma H, Beppu T, Okuda M, Onishi T. 2003. Survey 135 of *Ixodes* tick species on domestic. *Vet Parasitol.* 111(2-3): 231–239.
- Shimada Y, Inokuma H, Beppu T, Okuda M, Onishi T. 2003. Survey of *Ixodes* tick species on domestic. *Vet Parasitol.* 111(2-3):231–239.
- Taanman J. 1999. The mitochondrial genome: structure, transcription, translation and replication. Netherlands: Elsevier B.V.; p. 103–123.
- Thomas D, Burger A, Renfu Shao B, Stephen C, Barker A. 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(4):265–274.