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Complete mitochondrial genome sequence of *Dermacentor marginatus* (Acari: Ixodidae) isolated on Hainan Island, China: molecular characterization and phylogenetic implications

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Abstract

The complete mitochondrial genome sequence of *Dermacentor marginatus* isolated from Hainan Island, a tropical island in China, has recently been elucidated. This ornate sheep tick is a three-host species, meaning it requires feeding on different hosts throughout its larval, nymph, and adult stages. This broad host range contributes significantly to its role in the transmission of various pathogens including spotted fever group rickettsia. The mitochondrial genome of *D. marginatus* was sequenced and found to be a circular, double-stranded DNA molecule with a total length of 15,005 bp. The genome exhibited a guanine and cytosine (GC) content of 21.23%, reflecting its unique genetic composition. Annotation of the genome revealed the presence of 37 genes, comprising 22 tRNA genes, 13 mRNA genes as protein-coding genes, (PCGs) and two rRNA genes. Phylogenetic analysis placed *D. marginatus* closest to *D. silvarum* from Heilongjiang province in China, clustering with *D. sinicus* from Hubei province in China. *D. marginatus* also exhibited proximity to *D.* species from China and other countries forming a distinct cluster. The availability of the complete mitochondrial genome sequence of *D. marginatus* marked a notable advancement in deciphering the genetic diversity and evolutionary path of this tick species. The study concentrated on the mitochondrial genome of *D. marginatus* from Hainan Island, examining its genetic and phylogenetic features, and assessing genomic variations that may influence mitochondrial function and tropical adaptation. The investigation into the genetic adaptations of *D. marginatus* to its tropical niche yielded vital information for forecasting how tick populations may react to changes in climate and diverse environmental conditions.

Introduction

The ornate sheep tick, *Dermacentor marginatus* (Sulzer, 1776; Acari: Ixodidae), an obligate hematophagous ecto-parasite, depends on blood meals from vertebrate hosts for survival and reproduction.[1] As a three-host tick, it utilizes different hosts throughout its life cycle, facilitating the transmission and cycling of various pathogens. This species parasitizes a wide range of vertebrates, including small to medium-sized mammals during larval and nymph stages, and large mammals such as cattle, deer, dogs, and humans during adulthood.[2] *Dermacentor marginatus* is distributed across Europe, and adjacent Asian and African regions, primarily within the latitudes of 33° to 51°N,[3] with its southernmost limit in the northern Atlas Mountains (Morocco and Algeria) and northernmost boundary in northwestern Giessen, Germany.[4-6] The tick widespread presence is also noted in France, Italy and most European countries.[7-9] The completion of its life cycle is closely linked to climate change, with each developmental stage highly dependent on climatic conditions. Climatic suitability significantly influences tick population adaptation, determining conditions for survival, developmental trajectories and reproduction. Climate change has a profound influence on the distribution of ticks and the dynamics of pathogen transmission, exerting direct effects on their life cycles.[10] Variations in temperature and humidity directly impact the survival, activity scopes and even prompt ticks to migrate towards climates that are more conducive, establishing permanent residency in

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novel habitats. Climatic suitability not only shapes the habitat preferences of ticks but also seems to drive them into an adaptive evolution process, enabling them to better acclimate to the shifting climatic conditions. This adaptive evolution is crucial, as alterations in climatic conditions have modified the interactions between ticks and their hosts, consequently impacting the efficacy of pathogen transmission and the distribution patterns of genotypes.[11-12]

In the context of frequent analyses of tick distributions and their association with tick-borne diseases, *D. marginatus* serves as a potential vector for various tick-borne pathogens, notably those within the spotted fever group rickettsiae (SFGR). The SFGR, a globally distributed genus of intracellular bacteria, primarily infects a wide range of wild and domestic vertebrates via tick bites.[13] This group encompasses diverse diseases, such as rickettsial disease caused by *Rickettsia parkeri* and Rocky Mountain spotted fever induced by *R. rickettsii*.[14] In the Americas, notable SFGR-associated diseases include Rocky Mountain spotted fever, Mediterranean spotted fever caused by *R. conorii*, and *rickettsial pox* caused by *R. akari*.[15] In Africa, the tropical bont tick harbors *R. africae*, implicated as the primary transmitter of African tick-bite fever, affecting both local populations and tourists.[16] The West Indies, where *D. marginatus* was introduced from Africa, has witnessed an endemic spread of these pathogens, facilitated by inter-island livestock movement and the introduction of cattle egrets.[17] Historically, the Kimberley region of Western Australia reported SFGR infections among wartime troops, with recent discoveries of a novel SFGR member on Flinders Island and confirmations of Rickettsia infections in Peninsular Malaysia.[18-19] The emergence of tick-borne pathogens poses significant threats to human and livestock health, particularly in tropical regions of Africa, Asia and South America. This situation is exacerbated by the fact that climate change may expand the tick geographical range and activity cycle, especially in regions experiencing milder winters, which extends beyond tropical areas to temperate zones as well. This expansion is likely to lead to increased contact between ticks and potential hosts, thereby, enhancing the risk of pathogen transmission and replication. Such changes not only perpetuate the existing burden of tick-borne diseases in tropical regions but also threaten to introduce these diseases into new areas, posing significant health risks to humans and animals alike. This underscores the urgent need for continued surveillance and control measures to alleviate the burden of tick-borne diseases, taking into account the dynamic interplay between climate change and tick life cycles.

The mitochondrial genome, a circular, double-stranded DNA molecule typically 14 - 16 kb in length, is a reliable and efficient genetic marker for phylogenetic analyses. Its notable attributes include haploidy, matrilineal inheritance, a rapid evolutionary rate, and limited recombination.[20] Evolving five to ten times faster than nuclear sequences, the mitochondrial genome is particularly valuable for resolving species phylogenetic relationships.[21-22] Recently, the complete mitochondrial genome has proven to be a potent tool for characterizing genetic structure, elucidating molecular phylogeny and understanding intraspecific variation, thereby, advancing research in tick-borne disease control.

Hainan Island, located in China, is a tropical region distinguished by its unique tropical monsoon marine climate, featuring lush vegetation, high temperatures and persistent humidity. The examination of the mitochondrial genome of *D. marginatus*, collected from this island that provides optimal conditions for tick growth and reproduction, is of great significance.[23] This importance arises from the distinctive geographical and climatic characteristics of the region, which facilitate the exploration of genetic variations and adaptations unique to tropical environments. Such variations may not be fully encapsulated within existing genomic data originating from different climatic regions, thereby, highlighting the critical role of this research in bridging the existing knowledge deficit. The study was designed to delve into the intricate genetic and phylogenetic characteristics of *D. marginatus*, with a specific focus on its potential adaptations to environmental conditions. This endeavor was undertaken through a detailed examination of its mitochondrial genome, particularly from a tropical climate perspective. In the broader context, it is essential to recognize that over the long term, the process of tick adaptation under varying climatic conditions, such as those experienced in tropical regions, will elicit changes in their genetic diversity. Thus, our investigation not only aimed to further the exploration of *D. marginatus* and offer significant contributions to the domains of evolutionary biology and ecology, but also held immense significance for understanding the intrinsic connection between climate change and the life cycle of ticks.

Materials and Methods

Tick collection and morphological observation. *Dermacentor marginatus* ticks were sampled near Haikou City, Hainan Island, China (20° 2’ 35” N, 110° 23’ 07” E) using a drag-flag method with 1.00 m^2 white flannel in 2019. Ticks were carefully placed in 50.00 mL tubes using a brush for preservation. Morphological features were observed and documented with a VHX-5000 digital microscope (Keyence, Osaka, Japan) and a field-emission scanning electron microscope (S 4800; Hitachi, Tokyo, Japan). The tick species exhibits a unique morphological configuration, characterized by an elongated, ovate body that tapers progressively towards the anterior end, terminating in a broad, rounded posterior. On its dorsal surface, the cervical grooves are short and deep, and the scutum is adorned with irregular, dark punctations. The basis capituli displays a distinctive rectangular shape, notable for its relatively greater width compared to its length. Laterally positioned on the scutum are a pair of eyes, which are further complemented by a pattern of shimmering silver-gray enamel spots. At the ventral base of the idiosoma, 11 well-defined festoons are clearly evident. The second segment of the palps bears a small, short spur and the cornua are of short length. Furthermore, the first trochanter is equipped with a short posterior spur, while the lateral groove on the conscutum remains inconspicuous. Ventrally, Coxae I possess both external and internal spurs, separated by a medium-sized gap, with the external spur being slightly shorter than the internal one. Coxae II - IV are distinguished by their short external spurs, and Coxae IV is enlarged. These specific morphological attributes are of paramount importance in the identification and taxonomic classification of this particular tick species within the framework of acarological research.[24-27]

DNA extraction and sequencing. Genomic DNA was extracted using the MagPure Tissue DNA Kit (Magen Biotechnology, Guangzhou, China) and assayed on a 1.00% agarose gel. After quality check, the DNA was fragmented using ultrasound, purified, end-repaired, A-tailed, and ligated with sequencing adaptors. Fragment sizes were selected via agarose gel electrophoresis, followed by polymerase chain reaction amplification to create a library. The library was quality controlled and then sequenced at Genepioneer Biotechnologies using the NovaSeq 6000 platform (Illumina, San Diego, USA) generating 150 bp paired-end reads. Raw reads were quality controlled using fastp (version 0.23.4; OpenGene; Shenzhen, China), this process involved removing sequencing connectors, primer sequences, and low-quality sequences, which were defined as those with a quality score lower than quality score of 5 (Q5) or those containing unknown nucleotides with a content greater than 5.00%.[28] The remaining data, called clean reads, were evaluated using Q20 and Q30 values, which reflect sequencing accuracy (1.00 and 0.10% error rates, respectively). These values indicated the percentages of bases with quality scores greater than or equal to 20.00 and 30.00, respectively.

Genome assembly and annotation. Mitochondrial genome sequencing reads were mapped using bowtie2 (version 2.2.4; Johns Hopkins University, Baltimore, USA) with default parameters and assembled with SPAdes (version 3.10.1; St. Petersburg State University, St. Peters-burg, Russia) to obtain the complete circular genome.[29-30] Annotations were performed on the Mitos2 web server with parameters set as E-value 5, max overlap 100, and ncRNA overlap 100.[31] The gene structure map was visualized using OGDRAW (version 1.3.1; Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany).[32]

Genome sequence analysis. Dispersed repetitive sequences (DRS) in the mitochondrial genome are key for developing population and evolutionary markers. They were classified into four types including forward repeat sequences (F), reverse repeat sequences (R), complement repeat sequences (C), palindromic repeat sequences (P) and visualized using Vmatch (version 2.3.0; Bielefeld University, Bielefeld, Germany). DRS identification was set with a minimum size of 20 bp and a Hamming distance of three Codons show degeneracy, with usage varying among species and within genomes, known as relative synonymous codon usage (RSCU). The RSCU reflects the interplay of selection, mutation and genetic drift. The RSCU values, calculated using PhyloSuite (version 1.2.2; China Agricultural University, Beijing, China),[33] plotted in R Software package (version 3.6.1; R Development Core Team, Vienna, Austria) and compared to expected codon frequencies. A value > 1.00 indicates codon preference, with higher values showing stronger preference. In the mitochondrial genome, tRNAs are essential for protein synthesis. They typically have 73 - 90 nucleotides in a cloverleaf-like structure with four stems, three loops, and specific functional domains. The secondary structures of 22 tRNAs from *D. marginatus* were analyzed using tRNA scan-SE (version 1.21; University of California Santa Cruz, Santa Cruz, USA) and ARWEN (version 1.2; Uppsala University, Uppsala, Sweden).[34-35]

Phylogenetic exploration. Mitochondrial genome sequences were retrieved from GenBank® at National Center of Biotechnology Information (NCBI) and aligned using MAFFT (version 7.450; Osaka University, Osaka, Japan).[36] A phylogenetic tree was constructed from 19 tick sequences, with *Ornithodoros hermsi* and *Orectognathus rostratus* as outgroups. The alignment included new *D. marginatus* sequences and 10 *Dermacentor* species, plus three other Ixodidae genera: *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus*. The tree was built using RAxML (version 8.2.10; Heidelberg Institute for Theoretical Studies, Heidelberg, Germany) with the GTRGAMMA model and 1,000 bootstrap replications.[37]

Results

Sequencing data quality. A total number of 124 male ticks of *D. marginatus* were identified (Fig. 1). Sequencing data generated on the Illumina platform were archived in the Sequence Read Archive (SRA) of NCBI. (Bioproject: PRJNA1063922, BioSample: SAMN39413024, Run: SRR27592926). The clean data yielded 26, 501, 931 pair-end reads, totaling 7, 950, 579, 300 bases, with a GC content of 48.42%, Q20 of 97.52%, and Q30 of 93.49%.

Genome annotation. The mitochondrial genome of *D. marginatus* is a circular, double-stranded DNA molecule (Fig. 2A) that is 15,005 bp long with a 21.23% GC content.

It contains 37 genes: 22 tRNA genes, 13 mRNA genes and two rRNA genes. The 22 tRNA genes are distributed between the 5'-3' outer heavy chain (14 genes) and the 3'-5' medial light chain (eight genes). The 13 mRNA genes are located on the outer strand (nine genes) and inner strand (four genes). The two rRNA genes, encoding 12S (rrnL) and 16S (rrnS) ribosomal RNAs, are on the inner strand. Stop codons are found at trnL1-Ter, trnS2-Ter, and trnL2-Ter.

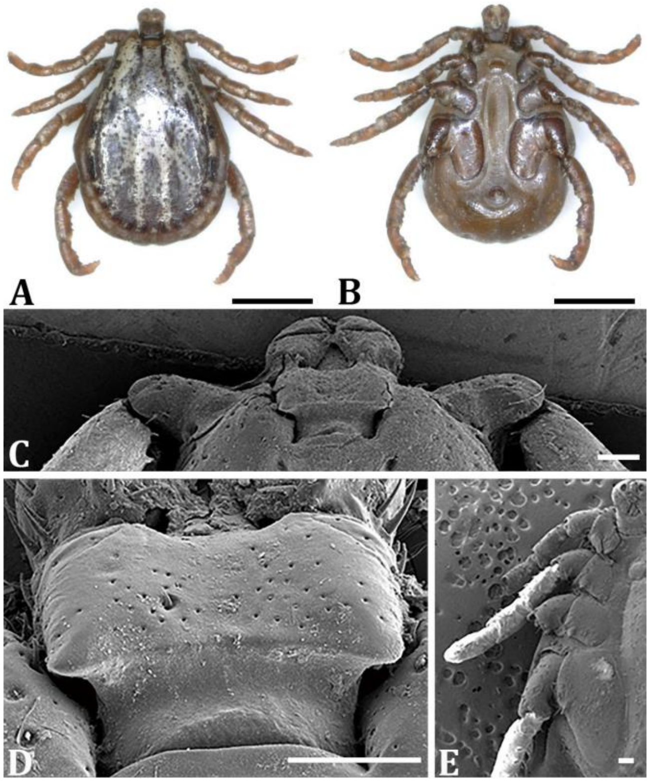

Fig. 1. Morphological characteristics of *Dermacentor marginatus*. **A)** Dorsal view, **B)** Ventral view, **C)** Capitulum and cervical grooves, **D)** Basis capituli and cervical grooves, and **E)** Coxae (Bars = 2,000 µm in A and B; 200 µm in C, D and E).

Sequence characterization. The mitochondrial genome sequence of *D. marginatus* had 2,316 DRS (Fig. 3). There were 580 F repeats, 622 P repeats, 645 R repeats, and 469 C repeats, accounting for 25.04, 26.86, 27.85, and 20.25% of the total DRS count, respectively. The DRS length varied from 20 to 263 bp, with 2,296 sequences being no more than 30 bp long, making up 99.14%. Only 20 DRS sequences were over 30 bp long, just 0.86% of the total. The high concentration of DRS within lengths below 30 bp indicated a high level of genome stability. Analysis of the RSCU revealed 28 codons with RSCU values exceeding one (Fig. 2B). Notably, 15 of these codons terminated in adenine (A), while 13 concluded with uracil (U). This distribution pattern underscored a distinct preference for codons terminating in either A or U within the mitochondrial genome.

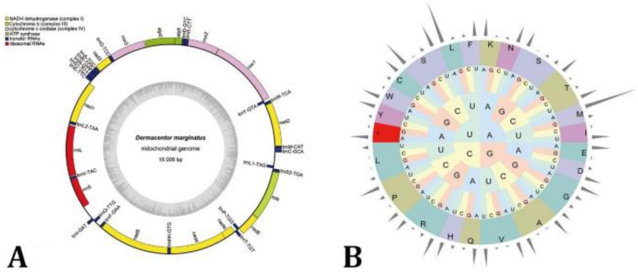

Fig. 2. Mitochondrial genome, dispersed repetitive sequences (DRS) and relative synonymous codon usage (RSCU). **A)** A detailed and annotated map of the mitochondrial genome of *Dermacentor marginatus*, depicting the locations of 37 genes, including 22 tRNA genes, 13 mRNA gene and two rRNA genes, and **B)** Graph of RSCU values. Codons with RSCU values above one, signifying preferential usage, were emphasized. A total number of 28 codons, including UAA, GCA, GCU, UGU, GAU, GAA, UUU, GGA, CAU, AUU, AAA, UUA, AUA, AAU, CCA, CCU, CAA, CGA, CGU, AGA, UCA, UCU, ACA, ACU, GUA, GUU, UGA, and UAU, were found to be over-represented, reflecting potential biases in codon usage within the mitochondrial genome.

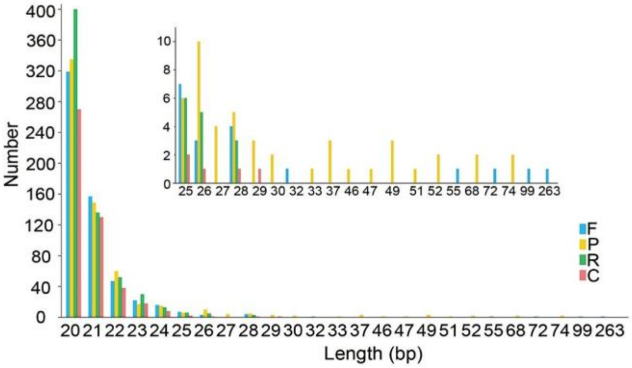

Fig. 3. Histogram of DRS length variations. The x-axis represented the length of DRS in bp, while the y-axis denoted the frequency of occurrence. The majority of DRS (99.14%) were confined to lengths not exceeding 30 bp, with a minor fraction (0.86%) exceeding this threshold, indicating a predominant concentration of DRS within a short length range, suggesting genomic stability. The smaller right panel magnifies the data with larger lengths (especially beyond 30 bp) from the left panel, clearly showing low frequencies for all categories and their differences at these longer lengths.

Fifteen of the 22 tRNA genes exhibited the characteristic cloverleaf structure (Fig. 4). Five tRNA genes were devoid of the thymidine-pseudouridine-cytidine stem (T psi C stem), and two lacked the dihydrouridine (D stem). Besides the canonical base pairings of guanine (G) with cytosine (C) and adenine (A) with uracil (U), these secondary structures harbored oscillating G-U pairs capable of stable chemical bonds between U and G.

Phylogeny. *Ornithodoros hermsi* and *Orectognathus rostratus*, both belonging to the family Argasidae and the genus Ornithodoros, served as outgroups. The *D. marginatus* sample was closest to *Dermacentor silvarum* (Heilongjiang, China) and *D. sinicus* (Hubei, China). It also showed proximity to other *Dermacentor* species, including *D. niveus* (Hubei, China), *D. marginatus* (Hubei, China), *D. reticulatus* (Russia), *D. rhinocerinus* (South Africa), *D. auratus* (Singapore), *D. andersonis* (USA), *D. albipictus* (USA), and *D. nitens* (Brazil), forming a distinct cluster. *D. marginatus* was phylogenetically distant from the genus *Haemaphysalis* and the genus *Rhipicephalus,* both of which belong to the family Ixodidae (Fig. 5).

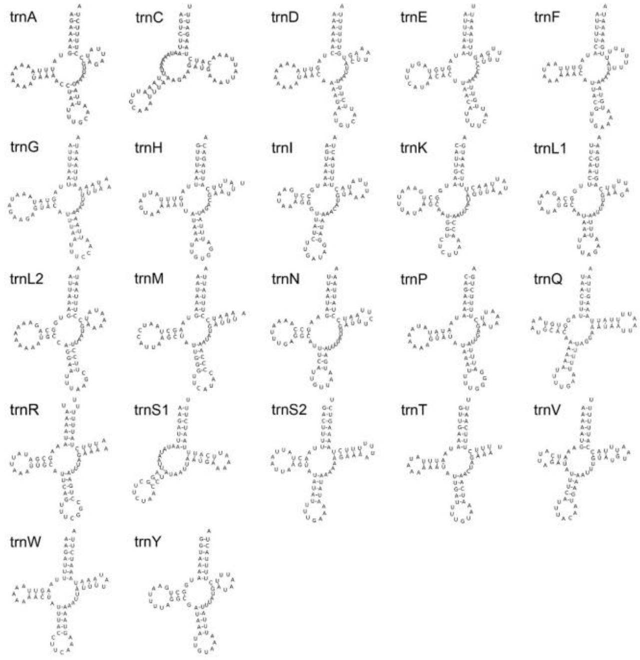

Fig. 4. Predicted secondary structures of tRNAs in the mitochondrial genome of *Dermacentor marginatus*. Fifteen tRNA genes exhibited the characteristic cloverleaf structure (trnD-Val, trnE-Phe, trnG-Ser, trnH-Val, trnI-Asp, trnK-Leu, trnL1-Ter, trnM-His, trnN-Val, trnQ-Leu, trnR-Ser, trnS2-Ter, trnT-Cys, trnV-Tyr, and trnW-Ser). Five tRNA genes (trnA-Cys, trnF-Glu, trnL2-Ter, trnP-Trp, and trnY-Val) were devoid of the T psi C stem, and two tRNA genes (trnC-Ala and trnS1-Ter) lacked the D stem.

Discussion

The completion of the mitochondrial genome sequencing of *D. marginatus* from Hainan Island offered a valuable advancement in the fields of ecology and genetics. These findings had profound implications for future epidemiological studies of *D. marginatus* ticks, as well as for the development of effective control strategies.

The mitochondrial genome of *D. marginatus* in this study was composed of 15,005 bp. A total number of five mitochondrial genomes of *D. marginatus* ticks were deposited in the GenBank® database of the NCBI website, with the accession numbers MK905212, NC062069, MT371801, OM368304, and OM368303. These genomes originate from Hubei province (the first three entries), Hebei province, and Xinjiang province, China, with respective lengths of 15,067 bp, 15,178 bp, 14,878 bp, 15,177 bp, and 15,178 bp. The GC content of the sequenced genome was 21.23%, aligning closely with the average GC content of approximately 20.00% observed across various tick species used in phylogenetic analyses. The *D. marginatus* genome encompassed 22 tRNA genes, two rRNA genes, and 13 PCGs, arranged in a manner similar to other tick species, suggesting a shared evolutionary ancestry.[38] This conserved gene composition indicates phylogenetic connections among ticks. The tRNA genes in the *D. marginatus* mitochondrial genome lacked the D stem, a characteristic commonly observed in numerous tick species.[39] Ticks display a wide array of tRNA secondary structures, including deletions in the D stem and T psi C stem. Truncated tRNA genes are prevalent in arachnid mitochondrial genomes. Phylogenetic analysis of tick mitochondrial genomes confirmed the distinct separation between hard ticks (Ixodidae) and soft ticks (Argasidae). Hard tick genera, including *Haemaphysalis*, *Hyalomma*, *Rhipicephalus*, and *Dermacentor*, clustered together, with *D. marginatus* showing close affinity to other *Dermacentor* species. The mitochondrial genome proved useful in resolving phylogenetic relationships within the tick population.

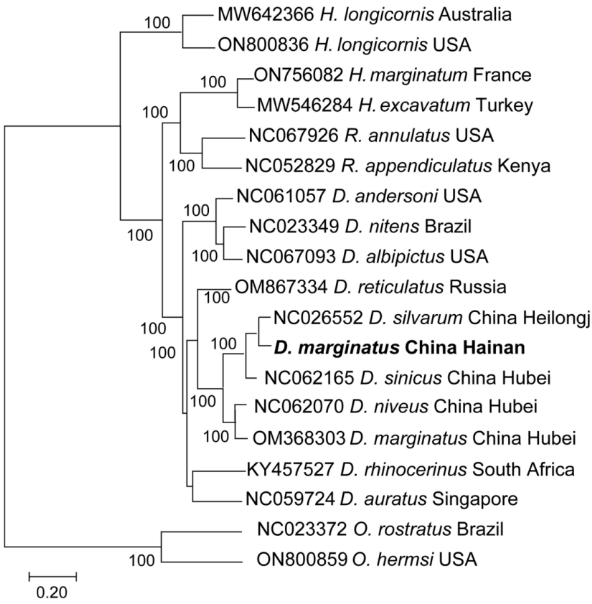

Fig. 5. Phylogenetic tree of the mitochondrial genome sequences retrieved from the GenBank® database. The tree revealed relationships among various tick species, with *Ornithodoros hermsi* and *Orectognathus rostratus* (Argasidae, Ornithodoros) as outgroups. *Dermacentor marginatus* showed the closest affinity to *D. silvarum* (Heilongjiang, China) and *D. sinicus* (Hubei, China), and clustered with other *Dermacentor* species from diverse locations, including *D. niveus* and *D. marginatus* (both Hubei, China), *D. reticulatus* (Russia), *D. rhinocerinus* (South Africa), *D. auratus* (Singapore), *D. andersoni* and *D. albipictus* (both USA), and *D. nitens* (Brazil). *D. marginatus* was phylogenetically distinct from the genera *Haemaphysalis* and *Rhipicephalus* (Ixodidae).

The detailed characterization of the mitochondrial genome of *D. marginatus* facilitated more precise species identification and phylogenetic analysis, which was a critical factor in the transmission of diseases. The genetic characteristics of the mitochondrial genome of *D. marginatus* in the tropical environment of Hainan Island, which may be associated with its adaptation to the unique tropical environment. These insights were crucial for risk assessments and surveillance strategies targeting tick-borne diseases on Hainan Island as well as in other regions sharing similar ecological conditions. Through the identification of genetic markers linked to disease transmission or vector competence, researchers were able to prioritize specific tick populations for more intensive monitoring and targeted control measures. Research into tick biology and evolution, taking into account the genetic adaptations of *D. marginatus* to its tropical environment, offered crucial information for anticipating how tick populations might react to shifts in climate and various environmental conditions. This comprehensive knowledge substantially improved the overall comprehension of tick ecology and was particularly vital in the context of global change, where changes in tick distribution and related disease risks were foreseeable.

Climate change plays a significant role in influencing the genetic structure of ticks, driving adaptive evolution in response to shifting climatic conditions. As previously mentioned, variations in temperature and humidity, which are key elements of climate change, directly affect multiple aspects of ticks including their survival, developmental trajectories, and activity scopes. Such impacts prompt tick populations to relocate towards more favorable climates and colonize new habitats. This migration and establishment in novel environments create selective pressures that act as a driving force for the genetic structure alteration of tick populations. Their genetic structure demonstrates adaptability to climate changes, allowing ticks to better acclimate to new environmental circumstances and improving their survival odds. This adaptability ensures that ticks can maintain their ecological functions and interactions with hosts even under the influence of climate change. As the climate continues to change, the genetic structure of ticks will likely continue to evolve, with advantageous genetic traits being selected and passed on, enabling ticks to persist and thrive in the face of a changing climate and its associated challenges to their survival and reproduction.

However, researching the genetic aspects of tick species, such as the mitochondrial genome of *D. marginatus* from Hainan Island, faces significant limitations due to the scarcity of available genetic data. The current gene databases contained only five sequences of *D. marginatus*, all of which were sourced from China. This limited dataset restricted the scope of comparative analyses and might not adequately represent the genetic diversity of the species across its entire distribution range. This limitation had several consequences for understanding *D. marginatus*. It might hinder the detection of subtle genetic variations crucial for elucidating the species' evolutionary history, population structure, and adaptability to environmental changes. The lack of diverse genetic data impeded the generalization of findings to broader geographical contexts, limiting the research applicability to other areas where *D. marginatus* was present. This was particularly concerning given the species potential involvement in disease transmission and the need for a comprehensive understanding of its biology and ecology.

To address these limitations, future research endeavors should strive to augment the genetic dataset by sampling a larger number of individuals from diverse populations of *D. marginatus* spanning various environments globally. Additionally, capturing a broader array of genetic traits through comprehensive sequencing efforts would markedly enhance our cognition of *D. marginatus*' biology, ecology, and its potential role in disease transmission. Such comprehensive efforts are essential to yield more robust and generalizable conclusions, especially in the context of understanding how climate change may continue to shape the genetic structure and adaptability of this tick species.

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Conflict of interest

The authors declare no conflict of interest.

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