Genetic diversity of hard ticks (Acari: Ixodidae) in the south and east regions of Kazakhstan and northwestern China

Yicheng Yang

Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China. Emergency Department, Shihezi City People's Hospital, Shihezi, City, Xinjiang Uygur Autonomous Region 832000, People's Republic of China.

Jin Tong

Department of Ultrasound, the First Affiliated Hospital of Medical College, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832008, People's Republic of China.

Hongyin Ruan

Department of Stomatology, School of Medicine, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China.

Meihua Yang

Department of Forestry, College of Agriculture, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China.

Chunli Sang

Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China.

Gang Liu

Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China.

Wurelihazi Hazihan

School of Animal Science and Technology, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China

Bin Xu

National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Centre for Tropical Diseases, Key Laboratory of Parasitology and Vector Biology of the Chinese Ministry of Health, Shanghai 200025, China.

Sándor Hornok

Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary.

Kadyken Rizabek

Department of Food Engineering, Kazakh National Agrarian University, Almaty province, Kazakhstan.

Kulmanova Gulzhan

Department of Food Engineering, Kazakh National Agrarian University, Almaty province, Kazakhstan.
Yuan-Zhi Wang (✉ wangyuanzhi621@126.com)
Shihezi University

Short report

Keywords: Genetic diversity, hard ticks, Kazakhstan, northwestern China

Posted Date: June 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-34664/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Version of Record: A version of this preprint was published at The Korean Journal of Parasitology on February 19th, 2021. See the published version at <https://doi.org/10.3347/kjp.2021.59.1.103>.

Abstract

Background

The Republic of Kazakhstan, located in Central Asia, ranks as the world's largest landlocked country, and borders five countries including China. There is a 1783 km long borderline between the South and East regions of Kazakhstan and Xinjiang Uygur Autonomous Region (XUAR, northwestern China). To date, there is no report on the genetic diversity of ticks in these regions.

Methods

During 2015–2019, a total of 4392 hard ticks (representatives of ten species) were collected from 605 animals (sheep, cattle, camels, dogs and hedgehogs) at 24 sampling sites belonging to 15 districts in southeastern Kazakhstan. After morphological identification, 213 specimens of these ticks were selected for molecular analyses. In addition, 157 hard ticks collected from sheep and camels between 2015 and 2018 in seven districts of XUAR were used for comparison. Following DNA extraction, a fragment of the mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene, ranging from 631 bp to 889 bp, was used to analyze genetic diversity among these ticks.

Findings:

Phylogenetic analyses indicated that i) five tick species including *Hyalomma detritum*, *Hyalomma asiaticum*, *Rhipicephalus turanicus*, *Dermacentor reticulatus* and *Haemaphysalis erinacei* from Kazakhstan clustered together with conspecific ticks from XUAR; ii) the phylogenetic separation of *Dermacentor marginatus* from Kazakhstan and XUAR was highly supported; and iii) *Rhipicephalus sanguineus* sensu lato from Almaty Oblast was more closely related to a specimen from Iran than to that from XUAR. The network diagram of haplotypes showed that iv) *Hy. asiaticum* from Almaty and Kyzylorda (Kazakhstan) together with that from Yuli County of XUAR constituted an ancestral haplogroup; and v) three lineages of *Rh. turanicus* (from Israel, Almaty and South Kazakhstan, as well as from Usu city, Ulugqat and Baicheng Counties of XUAR) might have originated from an ancestral lineage in Alataw city, XUAR.

Conclusions

These findings indicate that: (i) mitochondrial lineages of some tick species are shared between southern, eastern regions of Kazakhstan and northwestern China; (ii) common evolutionary origin of *Hy. asiaticum* and *Rh. turanicus* in these regions might be attributed to historical international trade and movements of wildlife; and (iii) certain tick species show clear differences between Kazakhstan and XUAR, either in

terms of abundance (*e.g. Hy. scupense, Hy. marginatum*) or exhibiting a phylogenetic split between these regions (relevant to *D. marginatus*).

Background

The Republic of Kazakhstan is located in Central Asia between 39°49' – 55°49' N and 46°28'–87°18' E, with its western part extending into Eastern Europe. Kazakhstan is the ninth largest country in the world with a total area of 2,727,300 km², also ranking as the world's largest landlocked country [1, 2]. It borders Russia, Kyrgyzstan, Turkmenistan, Uzbekistan and China [3]. Xinjiang Uygur Autonomous Region (XUAR), occupies one-sixth of China and borders eight countries, covering an area of approximately 1,660,000 km² [4]. The borderline between XUAR and the South and East regions of Kazakhstan is 1,783 km long [5]. The ecological environment, topography, climate and natural landscape are similar both in the XUAR and southeastern Kazakhstan [6].

Recently, fourteen tick species have been molecularly characterized in the border regions of XUAR [7]. At the same time, while at least thirteen tick species have been identified in Kazakhstan [8–11], their molecular characteristics are unknown. Here we report the genetic diversity of ticks in the South and East regions of Kazakhstan, in comparison with those from northwestern China.

Methods

Tick collection and morphological identification

During 2015–2019, a total of 4392 hard ticks were collected from 605 domestic animals (287 sheep, 210 cattle, 101 camels, 7 dogs) and 22 hedgehogs at 24 sampling sites belonging to 15 districts of five oblasts (East Kazakhstan, Almaty, Jambyl, South Kazakhstan and Kyzylorda) in the East and South regions of Kazakhstan (Fig. 1). After morphological identification, 213 of these ticks (representing all species) were selected for molecular analysis. In addition, 157 hard ticks collected from sheep and camels between 2015 and 2018 in seven districts of XUAR were used for comparison. The information on tick species, their host of origin and collection data (year and coordinates of location) are shown in **Appendix Table S1**.

Molecular Analysis Of Ticks

The genomic DNA was extracted from the selected 370 ticks individually using a tissue kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene, ranging from 631 bp to 889 bp, was used to analyze inter- and intraspecific genetic diversity of ticks. PCR primers and cycling conditions are shown in **Appendix Table S2**.

Phylogenetic Analysis Of Ticks

Sequences of the *cox1* gene were compared with GenBank data using BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) after manual editing. This dataset was resampled 1000 times to generate bootstrap values. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method. The best-fitting substitution model was determined with the Akaike Information Criterion using the ML model test implemented in MEGA 7.0 software [12, 13]. Genetic diversity was estimated as haplotype (h) and nucleotide diversity (π) indices with the programme DNAsp v5.10.1 [14]. True diversity (DIV_{mtDNA}) was assessed as the effective number of haplotypes on the sequence level [15]. Median-joining (MJ) networks were generated with the software Network v5.10.1 [16] to display the configuration of haplotypes and geographically localised phylogenetic haplogroups [17].

Results

Morphological and molecular identification revealed the presence of ten tick species in the five evaluated southeastern border oblasts of Kazakhstan, including *Hyalomma asiaticum*, *Hyalomma marginatum*, *Hyalomma scupense*, *Hyalomma detritum*, *Hyalomma anatolicum*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Rhipicephalus turanicus*, *Rhipicephalus sanguineus* and *Haemaphysalis erinacei*. Seven of these tick species (i.e., *Hy. asiaticum*, *Hy. detritum*, *Hy. anatolicum*, *D. marginatus*, *Rh. turanicus*, *Rh. sanguineus* and *Ha. erinacei*) were also represented in the sample group from XUAR, and could thus be used for molecular comparison. According to the latter, intraspecific sequence identities ranged from 96.27–100% using BLAST analysis of the *cox1* gene (shown in **Appendix Table S3**).

Phylogenetic analysis based on the *cox1* gene (Fig. 2) revealed that: i) *Hy. detritum* from East Kazakhstan (MN841460) clustered together with conspecific ticks from Turpan city of XUAR (KF583581) and Inner Mongolia Province of China (JQ737068); ii) *Hy. scupense* from Almaty (MN853164) and East Kazakhstan (MN853163) shared a clade with *Hy. scupense* from France and Russia (KX000638 and KU130633); iii) *Ha. erinacei* ticks from Almaty Oblast, Kazakhstan (MN841464) and Altau city of XUAR (KU364301) belonged to a different phylogenetic group than those from European countries; iv) *Hy. anatolicum* ticks from Jambyl Oblast (MN853167), Kazakhstan, Karshgar city of XUAR (KF583576) and Iran (KT920180) represented an ancestral clade to those from Iraq, India and Pakistan; v) *D. marginatus* from Qapqal County of XUAR (JX051151), East Kazakhstan (MN868592) and South Kazakhstan (MN868560) showed separation from *D. marginatus* from Alatau city of XUAR (KU364300) with high (100%) bootstrap support; and vi) *Rh. sanguineus* sensu lato from Almaty (MN862754) was more closely related to a specimen from Iran than to that from Altau city of XUAR (KU364307).

The network diagram of haplotypes showed that *Hy. asiaticum* from Almaty (MN892553) and Kyzylorda (MN961479) together with that from Yuli County of XUAR (KF527400) formed an ancestral haplogroup (H-2, in **Appendix File 1**). In addition, according to the present data, three lineages of *Rh. turanicus*, i.e. from Almaty and South Kazakhstan (MN853166 and MN841462, H-1 haplotype), XUAR (MF002579-

MF002581, H-3 haplotype), and Israel (KF219748, H-4 haplotype), originated from an ancestral lineage from Alataw city of XUAR (KY996841, H-2 haplotype) (**Appendix File 2**).

Discussion

The mitochondrial *cox1* gene, as the standard DNA barcode, is one of the most popular markers for population genetic and phylogeographic studies across the animal kingdom [18]. At the same time, it is the best choice for identification of tick species [19]. In the present study, five tick species from the South and East regions of Kazakhstan, including *Hy. detritum*, *Hy. asiaticum*, *Rh. turanicus*, *D. reticulatus* and *Ha. erinacei*, clustered together with conspecific ticks from XUAR. Nevertheless, different mitochondrial lineages were also shown to exist within some of these tick species (e.g. for *Hy. asiaticum*). The most likely reasons why specimens of diverse geographical origin shared common clades in the phylogenetic tree are as follows.

First, international trade of livestock and their products (e.g. skin and fur) may represent a historical link underlying genetic connection between tick populations in China and Kazakhstan. In this study, *Hy. asiaticum* in Almaty Oblast (MN892553), Kyzylorda (MN961479) and Yuli County of XUAR (KF527400) constituted one, ancestral haplogroup (H-2), while the H-1 haplogroup of *Hy. asiaticum* in South Kazakhstan (MN865123) originated from the H-3 haplogroup in XUAR (MK610453, MK307807 and MK213071). In addition, the network diagram of haplotypes indicated that *Rh. turanicus* in Alataw city (the largest economic port of XUAR), represents an ancestral lineage, which gave rise to three lineages. In particular, in the western direction it evolved as the H-1 haplogroup in Almaty (MN841462), Kazakhstan, whereas towards the eastern and southern directions it evolved as the H-3 haplogroup including ticks from Usu city (MF002580), Ulugqat County (MF002581) and Baicheng County (MF002579) in XUAR. Second, movements of wildlife and migratory birds are well-documented in these regions [20, 21], which might contribute to common genetic lineages of tick species. For instance, *Ha. erinacei* from hedgehog in Almaty, Kazakhstan and from marbled polecats in Altaw city of XUAR belonged to the same clade, which was well-separated from another phylogenetic group including specimens from Turkey and Romania.

However, the present study also has some limitations. First, tick species of the genus *Ixodes* were not available in Kazakhstan, and therefore could not be compared with those from XUAR. Second, *cox1* sequences of *Hy. scupense*, *Hy. marginatum*, and *D. reticulatus* were available from Kazakhstan, unlike corresponding data from XUAR, due to the scarce distribution of these tick species in the latter region. By themselves, these limitations also indicate that there are some differences in the occurrence of tick species and their abundance between XUAR and Kazakhstan. Therefore, it is an important task for the future to investigate further background factors of these differences, as well as to extend the scope of this study to ticks infesting domestic animals and wildlife in further countries neighboring XUAR, i.e. Russia, Kyrgyzstan, Turkmenistan, Uzbekistan, Afghanistan, Pakistan and India.

Abbreviations

XUAR: Xinjiang Uygur Autonomous Region; *cox1*: cytochrome *c* oxidase subunit I; *Hy. Asiaticum*: *Hyalomma asiaticum*; *Hy. marginatum*: *Hyalomma marginatum*; *Hy. Scupense*: *Hyalomma scupense*; *Hy. Detritum*: *Hyalomma detritum*; *Hy. Anatolicum*: *Hyalomma anatolicum*; *D. marginatus*: *Dermacentor marginatus*; *D. reticulatus*: *Dermacentor reticulatus*; *Rh. turanicus*: *Rhipicephalus turanicus*; *Rh. Sanguineus*: *Rhipicephalus sanguineus*; *Ha. Erinacei*: *Haemaphysalis erinacei*; ML: Maximum Likelihood; MJ: Median-joining.

Declarations

Acknowledgements

The authors thank the contributions by the staff at the Department of Anatomy, Physiology and Biochemistry, and the Department of Food Engineering, Kazakh National Agrarian University, Almaty, Kazakhstan.

Authors' contributions

YY, TJ and YW conceived and designed the study, and wrote the manuscript. YY, TJ, RH, YM, SC, LG, HW, XB, RK and GK performed the experiments, and analyzed the data. SH critically revised the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported in part by the National Key Research & Development Program of China (2018ZX10101002-007 and 2018ZX10101002-003) and National Natural Science Foundation of China (81960379 and 31960709).

Availability of data and materials

Data supporting the conclusions of this article are included within the article. The datasets used and analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China. ²Emergency Department, Shihezi City People's Hospital, Shihezi, City, Xinjiang Uygur Autonomous Region 832000, People's Republic of China. ³Department of Ultrasound, the First Affiliated Hospital of Medical College, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832008, People's Republic of China. ⁴Department of Stomatology, School of Medicine, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China. ⁵Department of Forestry, College of Agriculture, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China. ⁶School of Animal Science and Technology, Shihezi University, Shihezi, City, Xinjiang Uygur Autonomous Region 832002, People's Republic of China. ⁷National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Centre for Tropical Diseases, Key Laboratory of Parasitology and Vector Biology of the Chinese Ministry of Health, Shanghai 200025, China. ⁸Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary. ⁹Department of Food Engineering, Kazakh National Agrarian University, Almaty province, Kazakhstan.

References

1. Cherednichenko A. Estimating agricultural adaptation to climate change through cloud activation for northern Kazakhstan. In: Qi J, Evered KT, editors. Environmental problems of Central Asia and their economic, social and security impacts. Uzbekistan: Environmental Security; 2008. P. 183–190.
2. Yamanaka H, Murata K, Tabata R, Kawaguchi F, Sasazaki S, Yamamoto Y, et al. Kazakhstani Native Cattle Reveal Highly Divergent mtDNA From *Bos Taurus* and *Bos Indicus* Lineages With an Absence of *Bos Indicus* Y Chromosome. *Anim Sci J*. 2019;90(1):29-34.
3. Bismil'din FB, Shapieva ZZ, Anpilova EN. Current malaria situation in the Republic of Kazakhstan. *Med Parazitol (Mosk)*. 2001;(1):24-33.
4. Zhou M, Cao S, Sevinc F, Sevinc M, Ceylan O, Moumouni P F, et al. Molecular detection and genetic identification of *Babesia bigemina*, *Theileria annulata*, *Theileria orientalis* and *Anaplasma marginale* in Turkey. *Ticks Tick Borne Dis*. 2016;7(1):126-134.
5. Mihailovich SS, Isaevna MG. Land border delimitation of Republic of Kazakhstan: practical knowledge of border policy formation. *Eur Sci Rev*. 2018;(7–8): 202–204.
6. Hanapia S, Peng D, Junqi Y, Li C. Species diversity characteristics and conservation status in Xinjiang and Kazakhstan, China. *Anhui Agronomy Bull*. 2017;(16): 46–48.
7. Sheng J, Jiang M, Yang M, Bo X, Zhao S, Zhang Y, et al. Tick distribution in border Oblasts of Northwestern China. *Ticks Tick Borne Dis*. 2019;10(3): 665–669.
8. Nurmakhanov T, Sansyzbaev Y, Atshabar B, Deryabin P, Kazakov S, Zholshorinov A, et al. Crimean-Congo haemorrhagic fever virus in Kazakhstan (1948–2013). *Int J Infect Dis*. 2015;38:19–23.
9. Hay J, Yeh KB, Dasgupta D, Shapieva Z, Omasheva G, Deryabin P, et al. Biosurveillance in Central Asia: successes and challenges of tick-borne disease research in Kazakhstan and Kyrgyzstan. *Front Pub. Heal*. 2016;4:4.

10. Turebekov N, Abdiyeva K, Yegemberdiyeva R, Dmitrovsky A, Yeraliyeva L, Shapiyeva Z, et al. Prevalence of *Rickettsia* species in ticks including identification of unknown species in two Oblasts in Kazakhstan. *Parasit Vectors*. 2019;12(1):197.
11. Yashina L, Petrova I, Seregin S, Vyshemirskii O, Lvov D, Aristova V, et al. Genetic variability of Crimean-Congo haemorrhagic fever virus in Russia and Central Asia. *J Gen Virol*. 2003;84(Pt 5):1199–206.
12. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control Oblast of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol*. 1993;10(3): 512–526.
13. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*. 2016;33(7): 1870–1874.
14. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 2009;25(11): 1451–1452.
15. Jost L. The relation between evenness and diversity. *Diversity*. 2010;2(2): 207–232.
16. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol*. 1999;16(1): 37–48.
17. Wetjen M, Schmidt T, Schrimpf A, Schulz R. Genetic diversity and population structure of burbot *Lota lota* in Germany: Implications for conservation and management. *Fisheries Management and Ecology*. 2020;27(2): 170–184.
18. Avise J C, Ferguson MM. Molecular markers, natural history and evolution. *Systematic Biol*. 1995; 44(1): 117–119.
19. Lv J, Wu S, Zhang Y, Chen Y, Feng X, Yuan X. Assessment of four DNA fragments (*COI*, *16S rDNA*, *ITS2*, *12S rDNA*) for species identification of the *Ixodida* (Acari: Ixodida). *Parasit Vectors*. 2014;7(1): 93.
20. Wang YZ, Mu LM, Zhang K, Yang MH, Zhang L, Du JY, et al. A broad-range survey of ticks from livestock in northern Xinjiang: changes in tick distribution and the isolation of *Borrelia burgdorferi sensu stricto*. *Parasit Vectors*. 2015;8(1):449.
21. Guo LP, Mu LM, Xu J, Jiang SH, Wang AD, Chen CF, et al. *Rickettsia raoultii* in *Haemaphysalis erinacei* from marbled polecats, China-Kazakhstan border. *Parasit Vectors*. 2015;8(1):461.

Figures

Figure 1

A map of the study area. Left: Distribution of ticks in Kazakhstan and Xinjiang Uygur Autonomous Region (XUAR) in northwestern China. Right: tick species represented with different colors. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Phylogenetic analysis of tick species collected in Kazakhstan and Xinjiang Uygur Autonomous Region (XUAR) in northwestern China. The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000) based on cox1 sequences with MEGA7.0. Sequences of the tick species from Kazakhstan and XUAR of China obtained in this study are indicated by solid triangle (▲) and hollow triangle (Δ), respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AppendixFile2.docx](#)
- [AppendixFile1.docx](#)
- [AppendixTableS3.docx](#)
- [AppendixTableS2.docx](#)
- [AppendixTableS1.docx](#)
- [Graphicalabstract.jpg](#)