



Short communication

Tick distribution in border regions of Northwestern China

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ABSTRACT

Ticks are important vectors of emerging and re-emerging pathogens. The aim of this study was to determine tick species occurring in Xinjiang Uygur Autonomous Region (XUAR), especially on border regions. A total of 22,994 ticks (including 22,629 adults, 365 larvae and nymphs), belonging to six tick genera (i.e. *Dermacentor*, *Hyalomma*, *Rhipicephalus*, *Haemaphysalis*, *Ixodes* and *Argas*) and fourteen tick species, were collected from ten animal hosts in thirty-five counties (cities) in XUAR during 2011 - 2017. *Rhipicephalus turanicus*, *Dermacentor niveus*, *Hyalomma asiaticum* and *Dermacentor marginatus* were dominantly sampled from domestic animals while *Dermacentor nuttalli*, *Haemaphysalis punctata*, *Haemaphysalis concinna*, *Rhipicephalus sanguineus sensu lato*, *Dermacentor silvarum*, *Hyalomma scupense* and *Argas persicus* were sporadically found. Based on 16S rDNA, phylogenetic analyses showed that: i) *R. turanicus* genotypes in XUAR showed geographical separation, and belonged to clade I (major distribution in the Central Asian) rather than clade II (major distribution in the Mediterranean Basin); ii) *Ixodes kaiseri*, firstly sampled from Asian badgers (*Meles leucurus*), was in ancestral position compared to European tick species when combining COI haplotypes; and iii) *Haemaphysalis erinacei* from marbled polecats in China was a separate genotype compared with that in Mediterranean and Europe. Our findings suggest that geographical range plays a more important role than host-association in tick phylogeny, especially for *R. turanicus*, *I. kaiseri* and *H. erinacei*.

1. Introduction

China is located in the northern and eastern hemispheres, and can be characterized with diverse climatic conditions. The southern area is dominated by tropical weather, while the northern region has a sub-arctic climate, thus influencing the distribution of ticks in China (Zhang et al., 2016). Xinjiang Uygur Autonomous Region (XUAR), located in the hinterland of the Eurasian continent, it covers 1.66 million square kilometers and adjacent to eight countries with a 5,600-km long borderline. XUAR, located in the hinterland of the Eurasian continent, is geographically divided into two parts by Tianshan Mountain, namely

northern XUAR and southern XUAR. The former has a temperate continental arid and semi-arid climate, whereas the latter has a warm temperate continental arid climate (Zhang et al., 2016). The Junggar basin, situated in northern XUAR and between Altay and Tianshan Mountains, is composed of desert, Gobi Desert, saline beach and patched oases. The Tarim Basin, located in southern XUAR and between Tianshan, Kunlun Mountains and Altun Mountains, is composed of salinized land, a desert steppe and an oasis farmland. The distribution of tick species is mainly affected by various geographical habitats, climates and hosts (Zhang et al., 2016).

Up to now, 117 species of ticks were found in China, and more than

Abbreviations: BLAST, basic local alignment search tool; IUCN, International Union for Conservation of Nature; XUAR, Xinjiang Uygur Autonomous Region; COI, cytochrome c oxidase subunit I; MEGA, molecular evolutionary genetics analysis

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one third of these occurred in XUAR (Chen et al., 2010). Five tick species, i.e. *Ixodes persulcatus*, *Dermacentor nuttalli*, *Hyalomma asiaticum*, *Dermacentor marginatus*, and *Dermacentor niveus* (based on sampling free-living ticks), were dominant three decades ago (Kong et al., 1987). According to another survey (concerning fourteen counties or cities, northern region of XUAR), the most common tick species from livestock included *Hyalomma asiaticum asiaticum*, *D. nuttalli*, *D. marginatus*, *Haemaphysalis punctata* and *Rhipicephalus turanicus* (Wang et al., 2015). To date, there have been few comprehensive reports regarding the distribution and abundance of ticks from domestic animals and wildlife in XUAR, especially on border regions. Ticks were reported to transmit bacterial, viral and parasitic diseases to animals and humans (Shah et al., 2004; Wang et al., 2015; Li et al., 2015). Therefore, it is important to survey of the current geographical distribution, hosts and habitats of tick species in XUAR.

2. Materials and methods

2.1. Sampling area

During 2011–2017, an investigation on tick distribution in thirty-five counties (cities) of XUAR was carried out (shown in Fig. 1). The study area ranged between latitude 34°25′48″10″N and longitude 73°40′96″18″E. In accordance with the different geographical landscapes, 1–4 sampling sites were selected in each county or city. Geographic information regarding the tick habitats was shown in Supplementary Table 1.

2.2. Tick sampling

According to the annual peak activities of hard ticks, the sampling time was selected as late March to early April in southern XUAR, while late April to early May in northern XUAR. The soft ticks were sampled in late July to early August (Phulan and Bhatti, 1984). Parasitizing ticks

were sampled over the entire body of each pasture-based livestock or free-range poultry at different intervals under the owner agreements (Wang et al., 2015; Zhang et al., 2016). As to wildlives (marbled polecats, Asian badgers and common pipistrelle bats), their carcasses were submitted for postmortem examination to the Xinjiang Uygur Autonomous Region Wildlife Management Office, and then sent to our laboratory for scientific research. The ticks infestation on carcasses were directly collected, and immediately placed into screw-capped tubes containing several minute holes. Free-living ticks were sampled by flagging method or forceps directly from the ground (Zhang et al., 2016; Wang et al., 2015). The information regarding all of the collected specimens, including their loci, tick numbers, hosts and geographical habitats, were recorded in Supplementary Table 1.

This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2011-01).

2.3. Ticks identification

The collected ticks were identified by morphological characteristics using a recent description (Estrada-Peña et al., 2014; Walker et al., 2003; Tesgera et al., 2017). In particular, adult ticks were identified to genus based on their basis capituli, scutum and spiracular plates, while the identification of nymphs and larvae was primarily according to scutal dimensions, scutal-alloscutal setal length and medially serrate posterior palpal hairs (Estrada-Peña et al., 2014; Walker et al., 2003; Filippova, 1997; Hornok et al., 2017a,b). Pictures were taken with a stereomicroscope (LEICA M165 C). The representative tick specimens, with 1–4 ticks for each tick species at every sampling site, were used to analyse the genetic diversity. The genomic DNA was extracted from each individual tick using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China), following the manufacturer’s instructions. All the representative ticks were identified by 16S rDNA genetic marker, and partial tick species were further analysed by COI gene (Black and Piesman, 1994; Hornok et al., 2017a,b; Lv et al., 2014). The reference

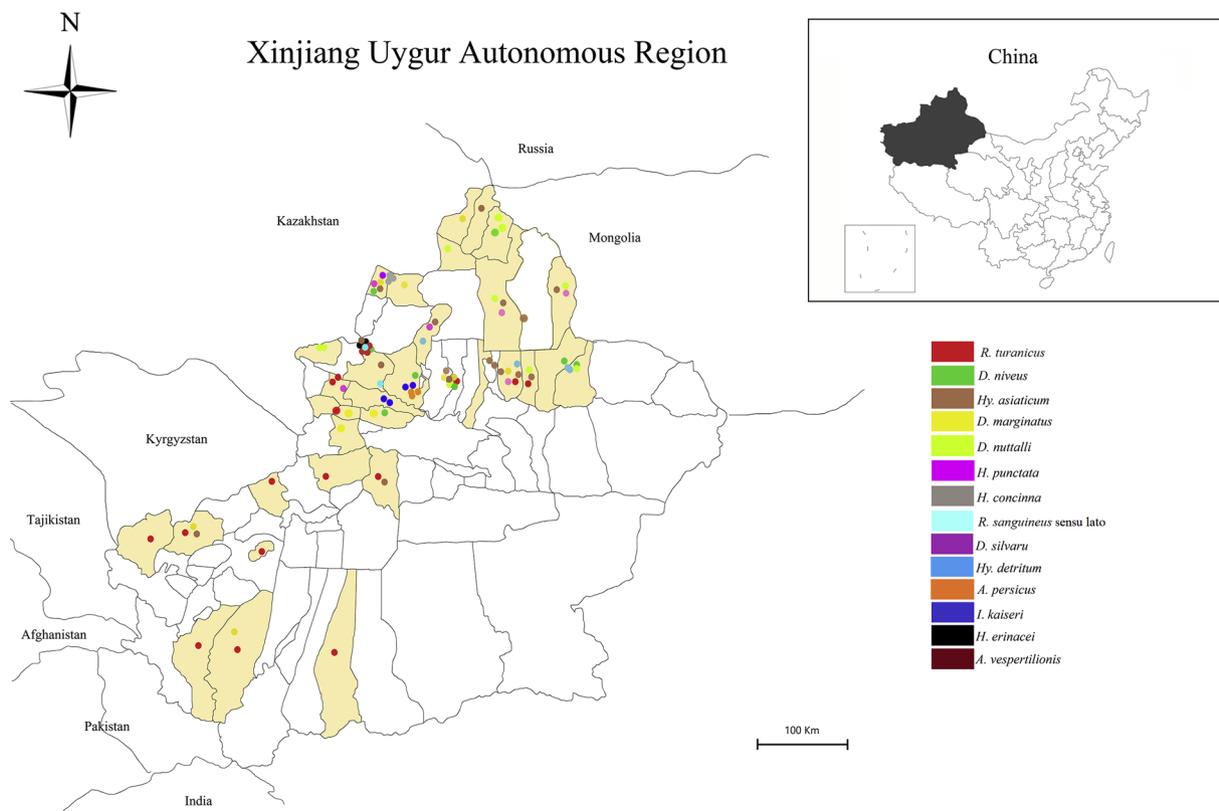


Fig. 1. A map of the study area. Left: the thirty-five surveyed counties or cities in XUAR and border countries. Right: the People’s Republic of China.

and our deposition sequences were adjusted manually and aligned with sequences in the GenBank database using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). This dataset was resampled 1000 times to generate bootstrap values. Phylogenetic relationships were conducted with 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 (Tamura and Nei, 1993; Kumar et al., 2016).

3. Results

A total of 22,994 ticks (22,629 adults, 365 larvae and nymphs), which belonged to six tick genera (i.e., *Dermacentor*, *Hyalomma*, *Ixodes*, *Argas*, *Rhipicephalus* and *Haemaphysalis*) and fourteen species, were collected from ten animal species in thirty-five counties (cities) in XUAR (shown in Supplementary Table 1). *R. turanicus*, *D. niveus*, *Hy. asiaticum* and *D. marginatus* sampled from domestic animals on border regions were dominant while *D. nuttalli*, *H. punctata*, *Haemaphysalis concinna*, *Rhipicephalus sanguineus* sensu lato, *Dermacentor silvarum*, *Hyalomma scupense* (as *Hyalomma detritum*) and *Argas persicus* were sporadically found. *Argas vespertilionis*, *Ixodes kaiseri* and *Haemaphysalis erinacei* were collected from common pipistrelle bats (*Pipistrellus pipistrellus*), Asian badgers (*Meles leucurus*) and marbled polecats (*Vormela peregusna*), respectively. Interestingly, *I. kaiseri* (including nine larvae and sixteen nymphs) was firstly found on Asian badgers ($n = 3$; 1135 m above sea level; 43°47'N, 82°30'E).

Comparative 16S rDNA sequence analysis shows that: i) the sequence divergence of *R. turanicus* in China, Afghanistan and Kyrgyzstan are 0–1.32%, whereas between Mediterranean countries and China are 3.53–4.84%; ii) all sequences from *I. kaiseri* ticks are closely related, exhibiting 0–0.3% sequence divergence; iii) Chinese *A. vespertilionis* shows 0–0.7% and 4.1–5.9% divergence between *A. vespertilionis* from European areas (Hungary, Romania and Italy), and Southeast Asia/East Africa areas (i.e. Vietnam and Kenya), respectively; iv) sequences of *H. erinacei* from China shows a range of 4.8–5.3% divergence from Italy, Hungary and Turkey.

Results of sequence alignments were confirmed by phylogenetic analyses (Fig. 2), 16S rDNA gene phylogenetic trees showed that: i) two different lineages of *R. turanicus* separate into clade I (including sequences of China, Kyrgyzstan and Afghanistan) and clade II (including sequences of Albania, Greece, Italy and Turkey), and six genotypes were found in this study; ii) *I. kaiseri* from Asian badgers was in ancestral position compared to European *COI* haplotypes (Supplementary Fig. 1), two-five nucleotide differences were found in comparison with *I. kaiseri* from Europe (accession No. MH389246 in this study); iii) combined with *COI* gene, *H. erinacei* from marbled polecats in China formed a sister group to those from the Mediterranean and Europe (accession No. KU364301 and KU364302 in this study).

In our study, a total of 116 reference sequences, all of the unique sequencing data from our study were deposited in the GenBank database. The details of sequence data are included in Supplementary Table 2.

4. Discussion

In this study, four tick species (*R. turanicus*, *D. niveus*, *Hy. asiaticum* and *D. marginatus*) predominated in samples collected from domestic animals along border regions, which was inconsistent with Kong et al. (1987), but consistent with Wang et al. (2015), with the exception of *H. punctata*. The latter might be related to different locations of tick sampling. In this study, the border counties (cities) were more focused. The variation in tick distribution in XUAR is attributed to a variety of factors like geographical habitat, temperature and humidity conditions (Wang et al., 2015). Here the habitats of alp, wetland, forest, semi-desert, Gobi Desert, lake, mountain and meadow were chosen during late March to early April, which might affect the dominant tick species.

Rhipicephalus turanicus is widely distributed in the desert and semi-desert areas of Tarim Basin and Junggar Basin in XUAR, China (Zhang et al., 2016). Together with 16S rDNA sequences of *R. turanicus* from China are grouped with sequences of *R. turanicus* sensu stricto from Afghanistan and Kyrgyzstan (clade I), which represent to *R. turanicus* s.s. ticks, whereas the sequences from the Mediterranean Basin and Europe were grouped into clade II (Chitimia Dobler et al., 2017; Nava et al., 2018; Li et al., 2017). The reason might be related to the various habitats. Afghanistan and Kyrgyzstan belong to semi-desert or desert regions, which is similar to XUAR. However, Albania, Greece, Italy and Turkey are situated in the Mediterranean Basin, with warm and humid climate in winter and dry and high-temperature in summer, which might have partially contributed to the evolution of different *R. turanicus* lineages compared to northwestern China-originated ones. This finding suggests that the evolution of different *R. turanicus* lineages is related to geographic distribution, which coincides with previous reports (Li et al., 2017; Dantas-Torres et al., 2013; Chitimia-Dobler et al., 2017; Nava et al., 2018). However, the genera of *Hyalomma* (*Hy. asiaticum* and *Hy. scupense*) and *Dermacentor* (*D. marginatus*, *D. niveus*, *D. silvarum* and *D. nuttalli*) do not reflect genetic diversity from different geographical regions.

Ixodes kaiseri is widespread in Europe and the Mediterranean, and infests European badgers, foxes, steppe polecats, raccoon dogs, and common hedgehogs according to a previous report (Hornok et al., 2017a,b). In this study, *I. kaiseri* was found in Nilka County (northern XUAR) from Asian badgers, of which were confirmed by 16S rDNA gene (accession no. MG021193). Phylogenetic analysis of the *COI* gene reflects that *I. kaiseri* from XUAR is in an ancestral position compared to European haplotypes (i.e. Hungary, Romania and Serbia) (Supplementary Fig. 1). In addition, *I. kaiseri* infesting *M. leucurus* showed 100% identity with reference sequences of the ticks infesting *S. undulatus* sampled in Jinghe County (northern XUAR) based on 16S rDNA and *COI* sequence (GenBank: MG656445 and MH279561). These findings support that the genetic diversity of *I. kaiseri* might be more related to geographical distribution rather than parasitic hosts, which is consistent to Klompen et al. (1996).

Argas vespertilionis is a cosmopolitan soft tick species with a wide distribution in the Old World from the Palaearctic to South Africa (Hoogstraal, 1956). In this study the sequence divergence between *A. vespertilionis* from China and Europe was less pronounced than between samples from China and Vietnam (reference sequences from Hornok et al. (2017a,Hornok et al., 2017b), suggesting that genetic exchange might be associated with latitude ranges (although a larger sample size from China is needed to draw final conclusion in this respect) (Lindgren et al., 2000). In addition, common pipistrelle bats (*Pipistrellus pipistrellus*), the host of *A. vespertilionis*, is widespread in Europe, the Middle-East, North Africa and Asia, which enhances the distribution of *A. vespertilionis* by the long-distance movement of bats (Hutterer et al., 2005; Bray et al., 2013).

Four genotypes of *H. erinacei* in this study were clustered into one clade based on the phylogenetic analysis of 16S rDNA gene (Fig. 2), which indicates different genetic lineages of *H. erinacei* exist even in same geographical location from marbled polecats. When further analyzing *COI* gene of *H. erinacei*, we found *H. erinacei* in XUAR was an dependent clade, and the sequence divergence ranged from 4.8% to 5.1% compared to that of Europe ticks (the data shown in Hornok et al., 2016), which further supported that *H. erinacei* from different geographical regions show genetic diversity (Hornok et al., 2016).

Multiple tick-borne pathogens, such as *Babesia vesperuginis* in *A. vespertilionis*, *Rickettsia raoultii* in *H. erinacei* and *Theileria ovis* in *R. turanicus*, have been reported in XUAR and China-Central Asia border region (Liu et al., 2018; Guo et al., 2015; Song et al., 2018). Here, *I. kaiseri* and *H. erinacei* ticks were respectively sampled from marbled polecats and Asian badgers, which were listed in the IUCN Red List of Threatened Species (<http://www.iucnredlist.org/>). In the future, it is important to systematically investigate tick-borne pathogens and tick-

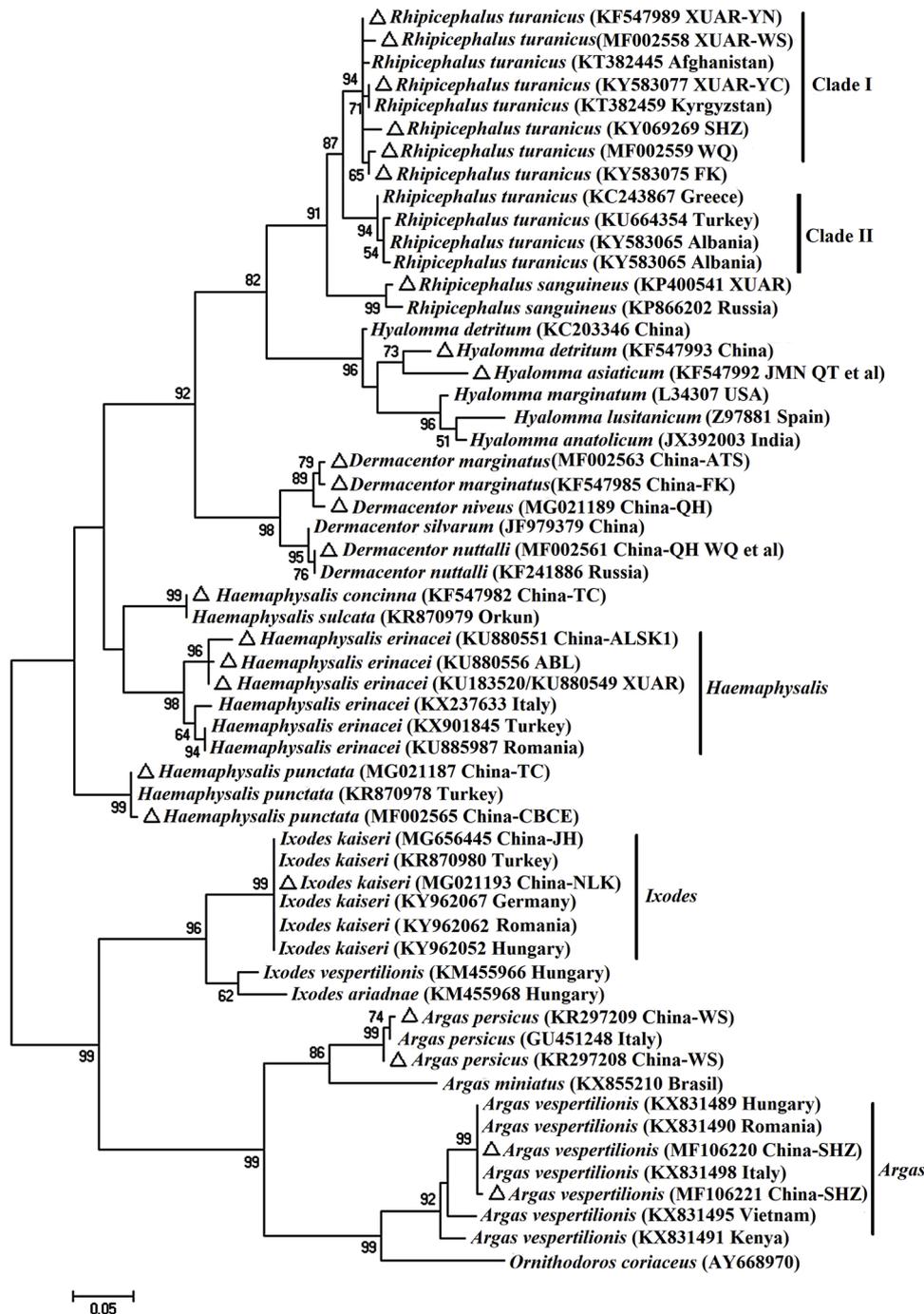


Fig. 2. The phylogenetic tree inferred from the 16S rDNA sequences of the representative tick specimens: sequences of the tick species obtained in this study are shown as (Δ). GenBank accession numbers are indicated in brackets. Branch lengths correlate to the number of substitutions inferred according to the scale shown.

borne diseases involving domestic animals and wildlife with the co-operation of Central Asian countries.

Conflict of interests

The authors declare that they have no conflict of interests.

Data availability

The datasets supporting the conclusions of this article are available in the GenBank (National Center for Biotechnology Information) [unique persistent identifier and hyperlink to datasets in <http://www.ncbi.nlm.nih.gov/genbank/>].

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

online version, at doi:<https://doi.org/10.1016/j.ttbdis.2019.02.011>.

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