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Genetic diversity of *Haemaphysalis qinghaiensis* (Acari: Ixodidae) in western China

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Abstract *Haemaphysalis qinghaiensis* as an endemic species in China mainly infests domestic animals and causes great harm to animals and humans in Northwestern plateau. However, there is no information about genetic diversity within the recently established populations of this tick species. Therefore, the present study analyzed the fragments of mitochondrial 16S rDNA, COI and the nuclear gene ITS1 of 56 *H. qinghaiensis* ticks across four regions of China which are main endemic areas of this species. Analysis showed 98.1–100% (16S rDNA), 97.9–100% (COI), 99.7–100% (ITS1) identity within individuals. For these sequences, 9, 15 and 8 haplotypes were found for 16S rDNA, COI and ITS1, respectively. Ticks from Yongjing were the most variable group, followed by Lintan, Huangyuan, and Tianzhu. Based on parallel analysis of the mitochondrial and nuclear genetic diversity of *H. qinghaiensis*, our results indicated that mitochondrial markers (especially COI) were much more useful than nuclear ITS for intraspecific genetic variability analysis.

Keywords *Haemaphysalis qinghaiensis* · Genetic polymorphism · China

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Introduction

Ticks are serious vectors of numerous human and animal pathogens, which are currently considered to be second only to mosquitoes. Associating tick haplotypes with its borne pathogens and the environment is very useful to control ticks and tick-borne disease. It is known *Haemaphysalis* is the second largest tick genus of the world, and the largest genus in China (Chen et al. 2010), many species of which closely related to humans. However, seldom studies of population genetic variation for *Haemaphysalis* tick species have been carried out. *Haemaphysalis qinghaiensis* Teng is a Chinese endemic species, and widely distributed in the western plateau (Teng and Jiang 1991; Chen et al. 2010, 2014a, b). This species is a three host tick, and often infest medium-large-sized domestic animals including sheep, goats, cattle, yaks, horses, mule and donkey, and sometimes humans. It was reported that *H. qinghaiensis* is an important vector of *Theileria* and *Babesia*, causing great harm to the animal husbandry and the health of animals and humans in Northwestern China (Guan et al. 2002, 2010; Li et al. 2007, 2009; Niu et al. 2017).

16S rDNA is frequently used to analyze the genetic variation within and among tick populations (Krakowetz et al. 2010, 2011; Dantastorres et al. 2013; Low et al. 2015; Arayaanchetta et al. 2015). Other genes such as 12S rDNA, COI, COIII, cytb, 18S rDNA, ITS1 and ITS2 are also used in genetic diversity studies for some tick species (Crosbie et al. 1998; Kempf et al. 2009; Tian et al. 2011; Gomezdiaz et al. 2012; Livanova et al. 2015). The aim of the present study was to determine the genetic polymorphisms of *H. qinghaiensis* from various ecoregions of China by parallel analyzing parts of COI, 16S rDNA, and ITS1 gene sequences.

Materials and methods

Tick collection

Huangyuan, Lintan, Yongjing and Tianzhu are main endemic areas of *H. qinghaiensis* in China. Their ecology and environment are similar to each other (continental climate and drought), but with different altitude. The percentage altitude of Tianzhu, Huangyuan, Lintan and Yongjing was 3500, 3100, 2825, and 1600 m, respectively. Therefore, *H. qinghaiensis* ticks were collected and sampled from these distinct locations with about 70,000 km^2 sampling area (Fig. 1, Table 1). Only ticks from Lintan were collected on vegetation by flagging, while others were captured from cattle. These specimens were identified by morphological characteristics (Chen et al. 2014a, b).

DNA isolation and amplification

The genomic DNAs of collected specimens (Table 1) were extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany). The primer sets of 16S rDNA, COI and ITS1 gene sequences were synthesized by Shanghai Sangon Biotechnology (Shanghai, China). Briefly, the primers 16SF (5′-CTGCTCAATGATTTTTTAAATTGCTGTGG-3′) and the reverse primer 16SR (5′-CCGGTCTGAACTCAGATCAAGT-3′) (Black and Piesman 1994) were used for 16S rDNA amplification, primers COIF1 (5′-AATTTA

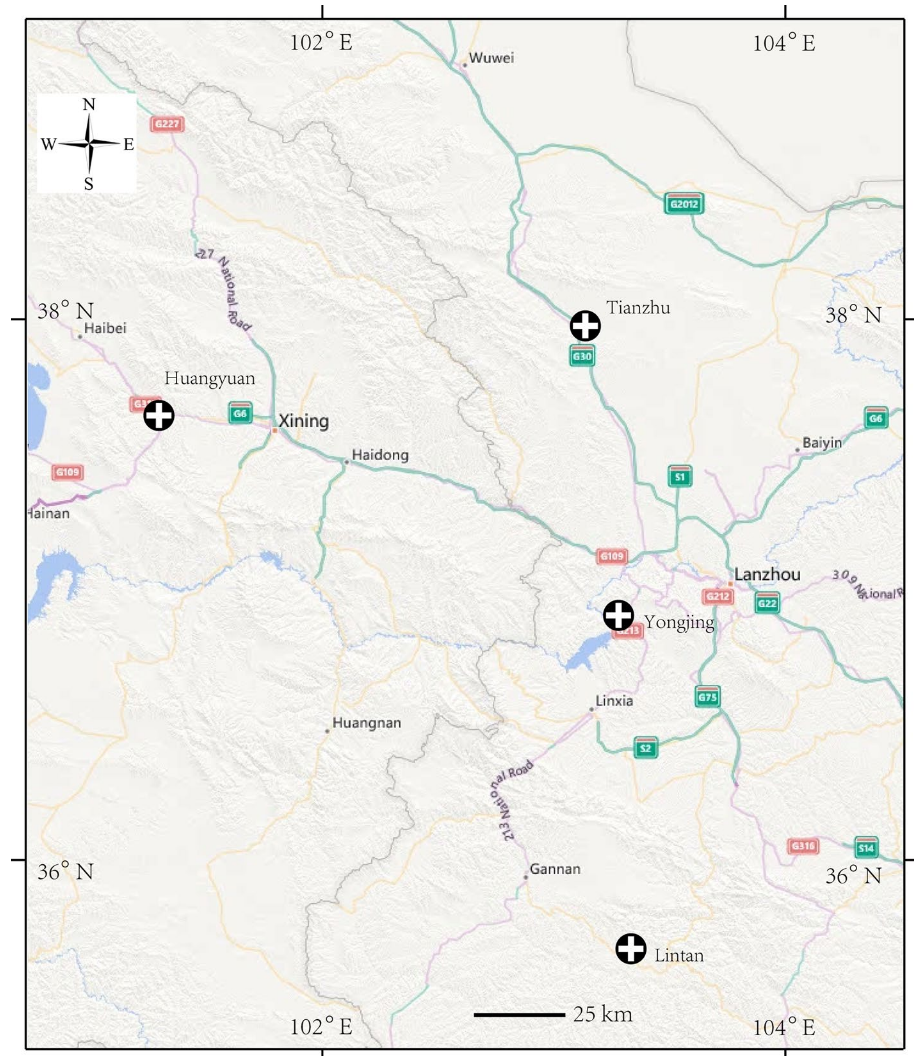

Fig. 1 Sampling sites of *Haemaphysalis qinghaiensis* ticks

CAGTTTATCGCCT-3′) and COIR (5′-CATACAATAAAGCCTAATA-3′) (Black and Piesman 1994) were used for COI gene amplification, and primers ITS1F (5′-TCATAA GCTCGCGTTGATT-3′) and ITS1R (5′-AGCTGGCTGCGTTCTTCAT-3′) (Chitimia et al. 2009) were used for ITS1 gene amplification. The PCR conditions were already described by Chen et al. (2012), and their products were about 450, 880 and 1400 bp for 16S rDNA, COI and ITS1, respectively. Negative controls (no template) were run in all PCR experiments. QIAGEN purification columns were used to purify 100 µl of PCR product according to the manufacturer's instructions. Both strands of the purified PCR product were sequenced twice by Shanghai Sangon Biotechnology.

Table 1 Source of tick specimens used in this study and their GenBank accession numbers

Collection localities	Specimens code	Developmental stage	16S rRNA	COI	ITS1
Lintan	LT1	Male	MF629876	MF981034	MF925303
Lintan	LT2	Female	MF629875	MF981035	
Lintan	LT5	Female		MF981036	
Lintan	LT6	Male		MF981037	
Lintan	LT7	Female	MF629874	MF981038	MF925304
Lintan	LT9	Male	MF629873	MF981039	MF925305
Lintan	LT10	Male	MF629872	MF981040	MF925306
Lintan	LT12	Female	MF629871	MF981041	MF925307
Lintan	LT15	Female	MF629870	MF981042	MF925308
Lintan	LT17	Male	MF629869	MF981043	
Lintan	LT19	Female	MF629868	MF981044	MF925309
Lintan	LT25	Female	MF629867	MF981045	MF925310
Lintan	LT26	Male	MF629866	MF981046	MF925311
Lintan	LT27	Male	MF629865	MF981047	
Lintan	LT28	Female	MF629864	MF981048	MF925312
Lintan	LT30	Male	MF629863	MF981049	MF925313
Lintan	LT32	Female	MF629862	MF981050	MF925314
Lintan	LT33	Male	MF629861	MF981051	MF925315
Lintan	LT41	Male	MF629860	MF981052	MF925316
Lintan	LT46	Female	MF629859	MF981053	MF925317
Lintan	LT48	Female	MF629858	MF981054	MF925318
Lintan	LT50	Male	MF629857	MF981055	MF925319
Lintan	LT53	Male	MF629856		MF925320
Yongjing	YJ1	Female	MF629848	MF981056	MF925328
Yongjing	YJ2	Female	MF629847	MF981057	MF925329
Yongjing	YJ3	Female	MF629846	MF981058	MF925330
Yongjing	YJ4	Female	MF629845	MF981059	MF925331
Yongjing	YJ5	Female	MF629844	MF981060	MF925332
Yongjing	YJ6	Female	MF622051		MF925333
Tianzhu	TZ1	Female	MF629855		MF925321
Tianzhu	TZ2	Female	MF629854	MF981061	MF925322
Tianzhu	TZ3	Male	MF629853	MF981062	MF925323
Tianzhu	TZ4	Female	MF629852	MF981063	MF925324
Tianzhu	TZ5	Female	MF629851	MF981064	MF925325
Tianzhu	TZ6	Female	MF629850	MF981065	MF925326
Tianzhu	TZ7	Female	MF629849	MF981066	MF925327
Huangyuan	HY1	Female	MF629896		MF801428
Huangyuan	HY2	Female	MF629895		MF801429
Huangyuan	HY3	Female	MF629894		MF925291
Huangyuan	HY4	Male	MF629893	MF981067	MF925292
Huangyuan	HY5	Male	MF629892		MF925293
Huangyuan	HY6	Female	MF629891	MF981068	MF925294
Huangyuan	HY7	Female	MF629890		MF925295
Huangyuan	HY9	Female	MF629889		MF925296

Table 1 (continued)

Collection localities	Specimens code	Developmental stage	16S rRNA	COI	ITS1
Huangyuan	HY10	Female	MF629888	MF981069	
Huangyuan	HY11	Female	MF629887		
Huangyuan	HY12	Female	MF629886	MF981070	MF925297
Huangyuan	HY13	Female	MF629885		
Huangyuan	HY14	Female	MF629884	MF981071	MF925298
Huangyuan	HY15	Female	MF629883	MF981072	MF925299
Huangyuan	HY16	Female	MF629882	MF981073	MF925300
Huangyuan	HY17	Female	MF629881	MF981074	MF925301
Huangyuan	HY18	Female	MF629880	MF981075	
Huangyuan	HY19	Female	MF629879		MF925302
Huangyuan	HY20	Female	MF629878		
Huangyuan	HY21	Male	MF629877		

Data analysis

Using the software BioEdit 5.09 (Hall 1999) edited the sequencing results and verified the nucleotide polymorphisms. The determined sequences were aligned with the CLUSTALX 2.0.11 software. The distribution of genetic variation between groups was analyzed by the Kimura two-parameter model, as implemented in MEGA 6 (Tamura et al. 2013). Haplotypes were defined by TCS 1.13 when they differed by at least one base pair including indels (Clement et al. 2000). Genealogical relationships among haplotypes of *H. qinghaiensis* were constructed using TCS 1.13 (Clement et al. 2000).

Results

In total, 56 adult ticks of *H. qinghaiensis* were collected from four distinct locations, namely Huangyuan (20), Lintan (23), Yongjing (6) and Tianzhu (7). In order to compare the genetic diversity of mitochondrial 16S rDNA and COI, and the nuclear gene ITS1 in parallel, each individual tick was amplified all of the three gene fragments, including 16S rDNA (54 sequences), COI (42 sequences) and ITS1 (45 sequences) (Table 1). The alignment of 16S rDNA, COI and ITS1 sequences resulted in a total of 444, 783 and 1386 data matrix (including gaps), respectively. The summary statistics for the three gene fragments are described in Table 2.

Analysis of mt 16S rDNA, COI, and the nuclear gene ITS1 sequences showed 98.1–100% (16S rDNA), 97.9–100% (COI), 99.7–100% (ITS1) identity within *H. qinghaiensis* individuals. For these sequences, 9, 15 and 8 haplotypes were found for 16S rDNA, COI and ITS1, respectively (Figs. 2, 3, 4). Using the Kimura two-parameter substitution analysis, the value of intra-specific genetic distances was 0–0.011, 0–0.02, 0–0.004 for 16S rDNA, COI and ITS1, respectively.

As little intraspecific differences of ITS1 between *H. qinghaiensis* ticks, a comparing species of *H. flava* (KJ156375) were also used for the analysis of sequence identity and

Table 2 Information for 16S rRNA, COI and ITS1 gene sequence fragments of *Haemaphysalis qinghaiensis*

Gene name	No. sampled individuals	Gene aligned fragment length (bp)	Average AT content (%)	Mutation sites	No. distinct haplotypes
16S rDNA	54	444	A: 38.2 T: 37.2	14	9
COI	42	783	A: 36.7 T: 30.1	32	15
ITS1	45	1386	A: 21.7 T: 21.9	10	8

inter-specific genetic distances in this study. The identity of ITS1 sequences between *H. flava* and *H. qinghaiensis* ticks was 97.7–97.9%, and their interspecific distance was 0.01–0.02. This interspecific difference of ITS1 sequences between *H. flava* and *H. qinghaiensis* is similar to intraspecific difference of 16S rDNA and COI sequences among *H. qinghaiensis* ticks, although the interspecific difference is larger than intraspecific difference for ITS1 analysis.

Of the 444 characters used for 16S rDNA analysis, 430 characters were constant and 14 were mutations (Tables 2, 3). The reconstruction of the network showed 9 detected 16S rDNA haplotypes (Fig. 2). The TCS spanning tree rooted *H. qinghaiensis* haplotype HQS1 as the ancestral haplotype. The two haplotypes (HQS1 and HQS2) combined 85% of all sequences which were found in most sampling sites. Additionally, HQS1 was found in all studied locations, whereas HQS4–HQS9 was unique to the sampling sites. The largest number of mutation sites was 5, and the Haplotypes were HQS4 (LT48) and HQS7 (YJ3) (Table 3). *Haemaphysalis qinghaiensis* from Yongjing (6 ticks) was the highest various group with 5 haplotypes, followed by that from Lintan (5 haplotypes in 21 ticks), Huangyuan (3 haplotypes in 20 ticks) and Tianzhu (1 haplotype in 7 ticks).

The aligned 42 COI derived gene sequences resulted in a 783 bp data matrix. Of these, 749 characters were constant and 32 were mutations (Tables 2, 4). The reconstruction of the network showed 15 detected COI haplotypes (Fig. 3). The TCS spanning tree rooted *H. qinghaiensis* haplotype HQC1 as the ancestral haplotype. There was no haplotype found in all studied locations, and HQC4–HQC15 was unique to the sampling sites. The two haplotypes of HQC1and HQC2 combined 64% of all sequences which were found in 3 sampling sites (Huangyuan, Lintan and Tianzhu), and 2 sampling sites (Lintan and Tianzhu), respectively. Additionally, *H. qinghaiensis* from Yongjing (5 ticks) was the highest various group with 5 haplotypes, and every of these haplotypes was unique. The largest number of mutation sites was 11 with the Haplotype HQC9 (LT50), and followed 9 with the haplotype HQC2 (LT1, LT5, LT6, LT9, LT10, LT12, LT19, LT28, LT41, LT46, TZ4, TZ5, TZ7) and HQC12 (YJ3) (Table 4).

The lengths of the aligned sequences of ITS1 were measured 1386 characters. Tables 2 and 5 show 1376 characters are constant and 10 are mutations. The reconstruction of the network showed 8 detected ITS1 haplotypes (Fig. 4). The TCS spanning tree rooted *H. qinghaiensis* haplotype HQI1 as the ancestral haplotype. The two haplotypes (HQI1 and HQI2) combined 87% of all sequences which were found in most sampling sites. Additionally, HQI1 was found in all studied locations, whereas HQI3–HQI8 was unique to the sampling sites. The largest number of mutation sites was 3 with the

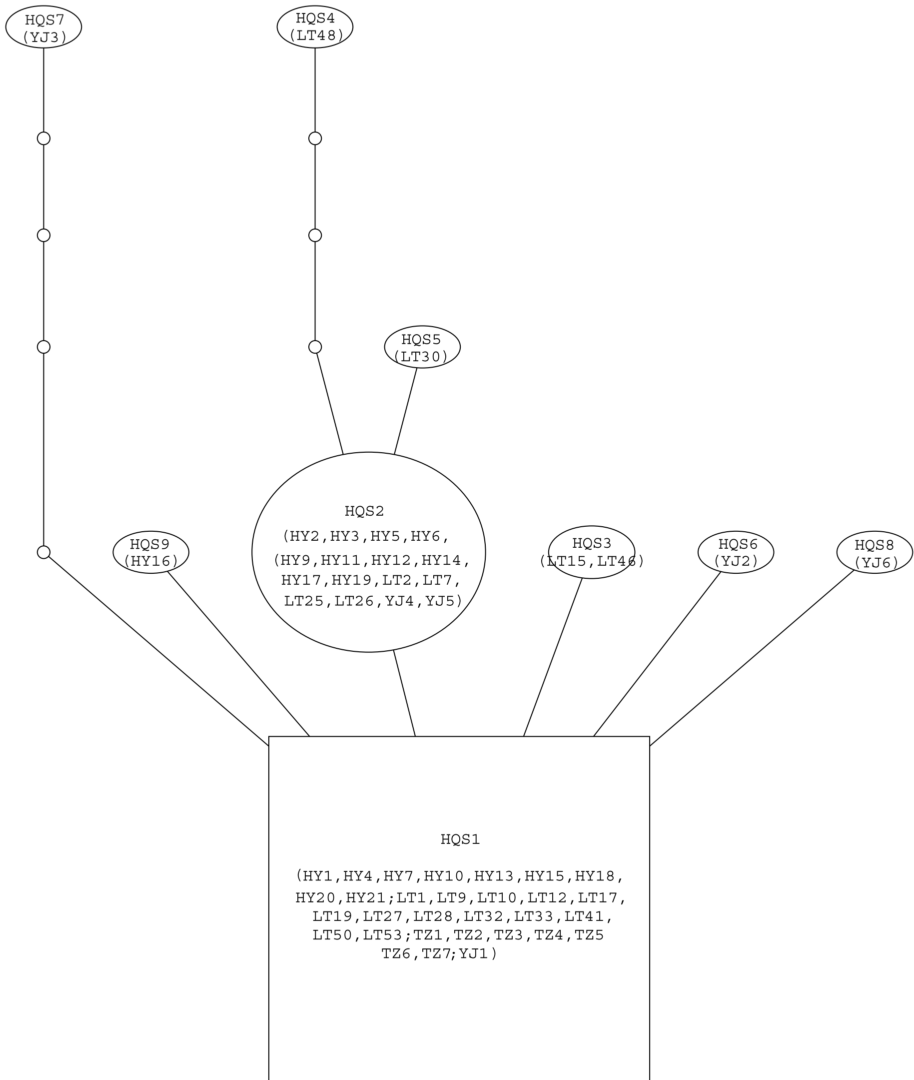

Fig. 2 Genealogical relationships of *Haemaphysalis qinghaiensis* based on nucleotide variability sites of 16S rDNA using TCS (Clement et al. 2000)

Haplotype HQI8 (YJ4), while each of most others was only one. In total, there were 2 haplotypes in 6 ticks from Yongjing, 1 haplotype in 7 ticks from Tianzhu, 3 haplotypes in 14 ticks from Huangyuan and 7 haplotypes in 18 ticks from Lintan.

The analysis of haplotypes of the three genes showed that *H. qinghaiensis* from Yongjing is the most genetically diverse population, and followed by Lintan, Huangyuan and Tianzhu (Table 6). Analysis of 16S rDNA, COI and ITS1 sequences showed 98.6–100% (98.5–99.7%, 99.7–100%), 98.6–100% (98.2–100%, 99.7–100%), 99.5–100% (99.7–100%, 99.8–100%) and 100% (98.7–100%, 100%) identity within *H. qinghaiensis*

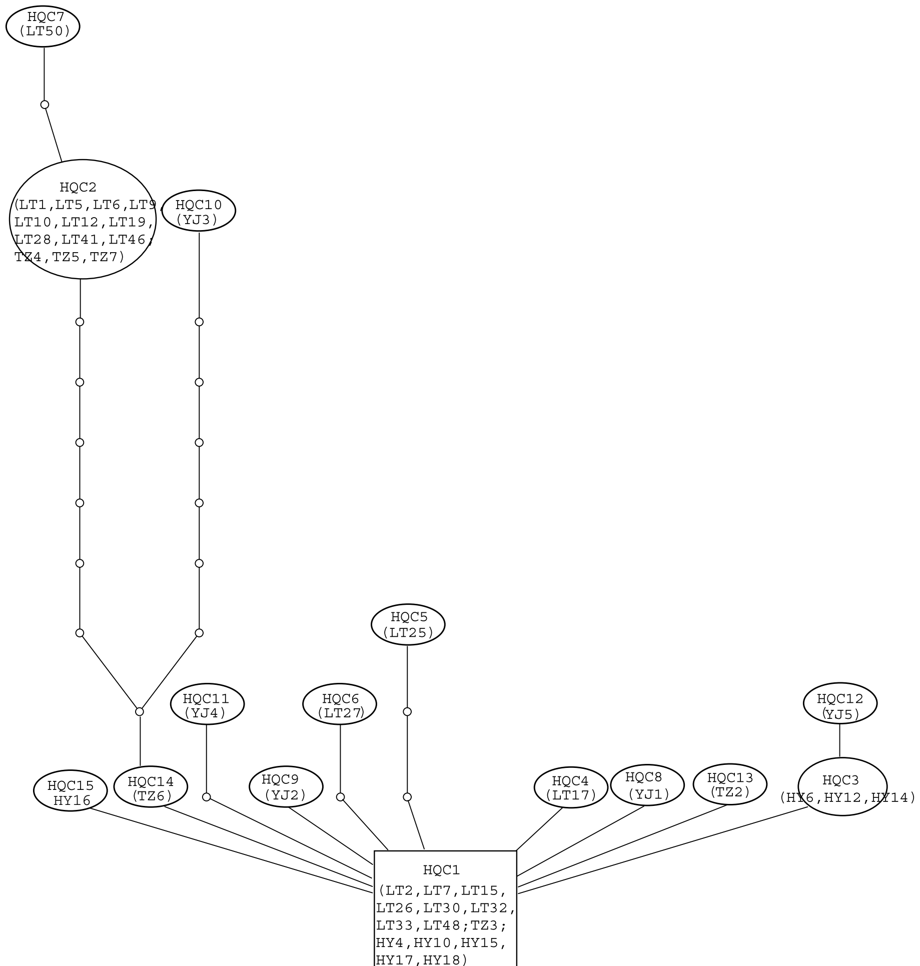

Fig. 3 Genealogical relationships of *Haemaphysalis qinghaiensis* based on nucleotide variability sites of COI using TCS (Clement et al. 2000)

individuals from Yongjing, Lintan, Huangyuan and Tianzhu, respectively. Meanwhile, analysis of mt 16S rDNA, COI, and the nuclear gene ITS1 sequences showed 98.1–100% (16S rDNA), 97.9–100% (COI), 99.7–100% (ITS1) identity within *H. qinghaiensis* individuals across the four regions. The genetic distance of 16S rDNA and ITS1 within and between the four regions was 0. Each genetic distance of COI within Yongjing, Lintan and Tianzhu was 0.01, while that within Huangyuan was 0. The genetic distance of COI between Yongjing and Huangyuan was 0, while others were 0.01.

Discussion

Up to now, seldom studies of genetic diversity for *Haemaphysalis* tick species have been carried out. *H. qinghaiensis* is an endemic species in western plateau of China and often

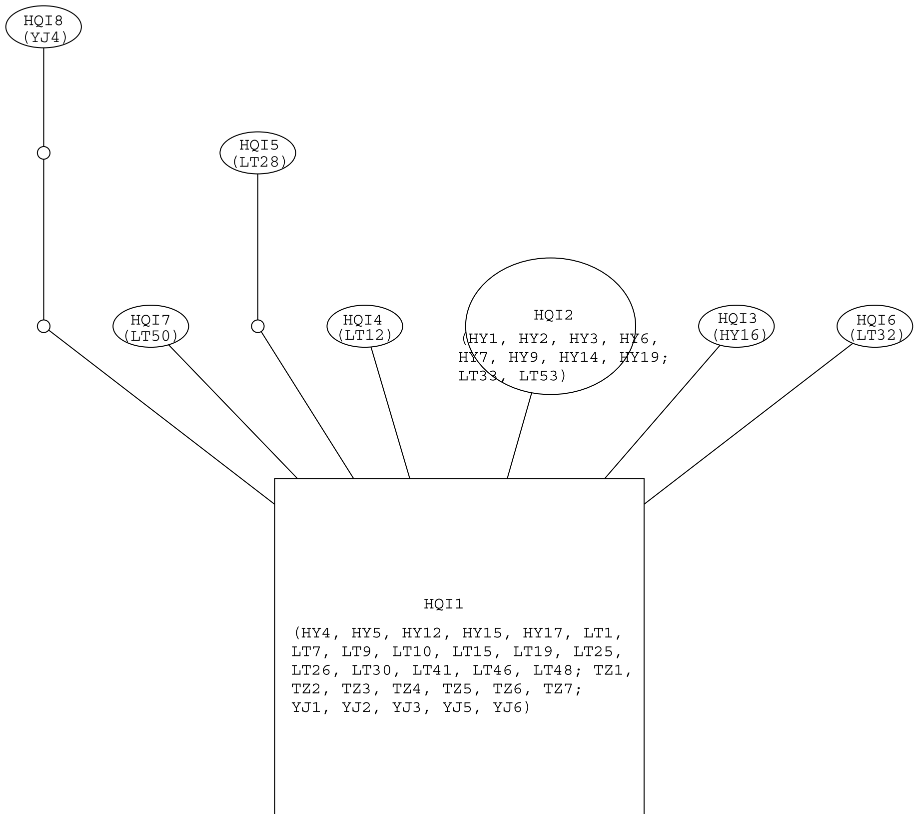

Fig. 4 Genealogical relationships of *Haemaphysalis qinghaiensis* based on nucleotide variability sites of ITS1 using TCS (Clement et al. 2000)

Table 3 DNA mutation sites based on 16S rDNA of *Haemaphysalis qinghaiensis*

	Mutation sites													
	50	187	189	190	192	193	194	195	200	204	205	333	396	397
HQS1	G	T	A	A	G	G	A	A	T	T	–	G	A	G
HQS2	–	–	–	–	–	–	–	–	A	–	–	–	–	–
HQS3	–	–	–	–	–	–	–	–	–	–	T	–	–	–
HQS4	–	–	–	–	T	T	C	C	A	–	–	–	–	–
HQS5	–	–	–	–	–	–	–	–	A	–	–	A	–	–
HQS6	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQS7	A	C	–	–	–	T	–	–	–	–	–	–	–	–
HQS8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQS9	–	–	–	–	–	–	–	–	–	–	–	–	–	A

Table 4 DNA mutation sites based on COI of *Haemaphysalis qinghaiensis*

	Mutation sites																															
	24	33	92	96	108	120	126	147	153	225	237	261	262	279	282	294	300	308	322	333	390	415	418	465	468	485	573	687	699	713	723	765
HQC1	C	A	G	G	G	A	A	G	G	C	G	A	A	T	T	C	T	T	A	T	A	G	A	G	T	A	A	G	T	T	C	T
HQC2	–	–	–	–	–	–	G	–	A	–	–	–	–	–	C	–	–	C	–	C	–	–	–	–	G	–	–	T	C	–	T	–
HQC3	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQC4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–
HQC5	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–
HQC6	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–
HQC7	–	–	–	–	–	–	G	–	A	–	–	G	–	–	C	–	–	C	–	C	–	A	–	–	G	–	–	T	C	–	T	–
HQC8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–
HQC9	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQC10	–	G	–	–	–	–	–	A	A	T	T	–	–	C	C	T	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–
HQC11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	G	–	–	–	–	–	–	–	–	–
HQC12	–	–	–	–	A	–	–	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQC13	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQC14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HQC15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C

Table 5 DNA mutation sites based on ITS1 of *Haemaphysalis qinghaiensis*

	Mutation sites									
	618	622	644	852	853	892	895	988	1375	1386
HQI1	T	A	A	A	T	T	A	C	A	A
HQI2	–	–	–	–	–	–	–	A	–	–
HQI3	–	–	–	–	–	C	–	–	–	–
HQI4	C	–	–	–	–	–	–	–	–	–
HQI5	–	–	–	–	–	–	G	–	–	G
HQI6	–	–	–	–	C	–	–	–	–	–
HQI7	–	G	–	–	–	–	–	–	–	–
HQI8	–	–	G	G	–	–	–	–	G	–

infest domestic animals (Teng and Jiang 1991; Chen et al. 2010, 2014a, b). This study was first to analyze the genetic diversity of mitochondrial 16S rDNA, COI and nuclear ITS1 fragments of *H. qinghaiensis* ticks in parallel. *Haemaphysalis qinghaiensis* ticks from different regions are hard to be identified from one population to another by morphological and molecular features. However, the genetic polymorphism of *H. qinghaiensis* from the four regions is different from each other.

In conclusion, most ticks were divided into the first two haplotypes of 16S rDNA, COI and ITS1, and the percentage was 85, 64 and 87%, respectively. The large percentage of singleton haplotypes (6/9 for 16S rDNA, 12/15 for COI, 6/8 for ITS1), and consistency of singleton and multiple haplotypes across the ecoregions indicated that these *H. qinghaiensis* populations were intermixing among the sampling areas (Figs. 2, 3, 4). This was also reported in other tick species (Trout et al. 2009, 2010; Kulakova et al. 2014). In this study, the percentage altitude of sampling area namely Tianzhu, Huangyuan, Lintan and Yongjing was 3500, 3100, 2825, and 1600 m, respectively. Interestingly, our study identified *H. qinghaiensis* ticks from Yongjing as the most genetically diverse population, and followed by Lintan, Huangyuan and Tianzhu. The region of Yongjing had 5 haplotypes for both of 16S rDNA and COI, of which 4 and 5 were unique, respectively. The sequences of most ticks from Tianzhu were identical to each other and to ancestral haplotype, especially for 16S rDNA and COI sequences. The reason of the different genetic polymorphism of *H. qinghaiensis* may be due to the height of altitude. Comparing to ticks at high altitude, the activity of ticks is more frequently at low altitude for *H. qinghaiensis*. Additionally, Yongjing located convenient with frequent livestock mobility, which might cause the frequent exchange of genes for ticks. Meanwhile Tianzhu is a much more out-of-the-way and backward place. Additionally, there was no relationship between genetic diversity and sex.

The 16S rDNA, COI and ITS1 sequences of *H. qinghaiensis* individuals revealed 98.1–100, 97.9–100, 99.7–100% identity to each other, respectively. Similar results were reported for other tick species: 99–100% (16S rDNA) for *H. longicornis* (Chen et al. 2015), 99.3–100% (16S rDNA and COI) for *Dermacentor everestianus* (Chen et al. 2014a, b). The intraspecific distance between *H. qinghaiensis* ticks was 0–0.011 (16S rDNA), 0–0.02 (COI), 0–0.004 (ITS1), which was comparable with results of 16S rDNA obtained for *H. concinna* (0.001), *H. longicornis* (0–0.001) and *H. punctata* (0.007) (Zhang and Zhang 2014; Chen et al. 2015). Crosbie et al. (1998) evaluated the intraspecific variation in 16S rDNA of 11 specimens of *D. hunter* and found that except for 4 ticks differed by a single base other 7 ticks were identical to each other. Similar to this study of *H. qinghaiensis*,

Table 6 Haplotypes of *Haemaphysalis qinghaiensis* of 16S rDNA, COI and ITS1 genes from different locations

Gene name	haplotype	Tick strain	Specimen source
16S rDNA	HQS1	HY1, HY4, HY7, HY10, HY13, HY15, HY18, HY20, HY21, LT1, LT9, LT10, LT12, LT17, LT19, LT27, LT28, LT32, LT33, LT41, LT50, LT53, TZ1, TZ2, TZ3, TZ4, TZ5, TZ6, TZ7, YJ1	Huangyuan (9), Lintan (13), Tianzhu (7), Yongjing (1)
16S rDNA	HQS 2	HY2, HY3, HY5, HY6, HY9, HY11, HY12, HY14, HY17, HY19, LT2, LT7, LT25, LT26, YJ4, YJ5	Huangyuan (10), Lintan (4), Yongjing (2)
16S rDNA	HQS 3	LT15, LT46	Lintan (2)
16S rDNA	HQS 4	LT48	Lintan (1)
16S rDNA	HQS 5	LT30	Lintan (1)
16S rDNA	HQS 6	YJ2	Yongjing (1)
16S rDNA	HQS 7	YJ3	Yongjing (1)
16S rDNA	HQS 8	YJ6	Yongjing (1)
16S rDNA	HQS 9	HY16	Huangyuan (1)
COI	HQC1	LT2, LT7, LT15, LT26, LT30, LT32, LT33, LT48, HY4, HY10, HY15, HY17, HY18, TZ3	Lintan (8), Huangyuan (5), Tianzhu (1)
COI	HQC 2	LT1, LT5, LT6, LT9, LT10, LT12, LT19, LT28, LT41, LT46, TZ4, TZ5, TZ7	Lintan (10), Tianzhu (3)
COI	HQC 3	HY6, HY12, HY14	Huangyuan (3)
COI	HQC 4	LT17	Lintan (1)
COI	HQC 5	LT25	Lintan (1)
COI	HQC 6	LT27	Lintan (1)
COI	HQC 7	LT50	Lintan (1)
COI	HQC8	YJ1	Yongjing (1)
COI	HQC 9	YJ2	Yongjing (1)
COI	HQC 10	YJ3	Yongjing (1)
COI	HQC11	YJ4	Yongjing (1)
COI	HQC 12	YJ5	Yongjing (1)
COI	HQC 13	TZ2	Tianzhu (1)
COI	HQC 14	TZ6	Tianzhu (1)

Table 6 (continued)

Gene name	haplotype	Tick strain	Specimen source
COI	HQC 15	HY16	Huangyuan (1)
ITS1	HQI1	HY4, HY5, HY12, HY15, HY17, LT1, LT7, LT9, LT10, LT15, LT19, LT25, LT26, LT30, LT41, LT46, LT48, TZ1, TZ2, TZ3, TZ4, TZ5, TZ6, TZ7, YJ1, YJ2, YJ3, YJ5, YJ6	Huangyuan (5), Lintan (12), Tianzhu (7), Yongjing (5)
	HQI2	HY1, HY2, HY3, HY6, HY7, HY9, HY14, HY19, LT33, LT53	Huangyuan (8), Lintan (2)
	HQI3	HY16	Huangyuan (1)
	HQI4	LT12	Lintan (1)
	HQI5	LT28	Lintan (1)
	HQI6	LT32	Lintan (1)
	HQI7	LT50	Lintan (1)
	HQI8	YJ4	Yongjing (1)

intraspecific variation in COI of *H. longicornis* was 0–0.027, which was also further than that of 16S rDNA (Lu et al. 2013).

Analysis of 16S rDNA, COI and ITS1 sequences showed 98.6–100% (98.5–99.7%, 99.7–100%), 98.6–100% (98.2–100%, 99.7–100%), 99.5–100% (99.7–100%, 99.8–100%) and 100% (98.7–100%, 100%) identity within *H. qinghaiensis* individuals from Yongjing, Lintan, Huangyuan and Tianzhu, respectively. Meanwhile, analysis of mt 16S rDNA, COI, and the nuclear gene ITS1 sequences showed 98.1–100, 97.9–100, 99.7–100% identity within *H. qinghaiensis* individuals across the regions, respectively. The genetic distance of 16S rDNA and ITS1 within and between the four regions was 0. Each genetic distance of COI within Yongjing, Lintan and Tianzhu was 0.01, while that within Huangyuan was 0. From the analysis above, the differences within each region is not larger than those across the regions.

In this study, an aligned 1386-bp segment of nuclear gene ITS1 used for analysis with the results of 10 mutation sites and 8 haplotypes, while an aligned much smaller segment of 16S rDNA (444) and COI (783) used for analysis with the results of more mutation sites (14 and 32) and haplotypes (9 and 15). The intraspecific variation of ITS1 was also much more conserved (0–0.004) than that of 16S rDNA (0–0.011) and COI (0–0.02). Beati et al. (2012) analyzed the genetic variability among *Amblyomma variegatum* ticks, and found that the ITS2 fragment consisted of only uninformative single nucleotide mutations. Similar results were also found by (Kulakova et al. 2014). They evaluated the intraspecific variation in 16S rDNA and ITS2 of 11 specimens of *D. nuttalli* and found that the genetic distance was 0.007 and 0.002, respectively. In conclusion, based on our parallel analysis of the mitochondrial and nuclear genetic diversity of *H. qinghaiensis* and reported studies of other tick species, mitochondrial markers (especially COI) should be much more useful than nuclear ITS1 and ITS2 for intraspecific genetic variability analysis.

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