



Spotted Fever Group *Rickettsia* Infecting Ticks (Acari: Ixodidae), Yak (*Bos grunniens*), and Tibetan Sheep (*Ovis aries*) in the Qinghai–Tibetan Plateau Area, China

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The Qinghai–Tibet Plateau Area (QTPA) has a complex natural ecosystem, causing a greatly increased risk of spreading various tick-borne diseases including rickettsial infections, which are regarded as one of the oldest known vector-borne zoonoses. However, the information of one of its pathogen, spotted fever group *Rickettsia* (SFG *Rickettsia*), is limited in tick vectors and animals in this area. Therefore, this study focused on the investigation of SFG *Rickettsia* in tick vectors, yaks (*Bos grunniens*), and Tibetan sheep (*Ovis aries*) in the QTPA. A total of 1,000 samples were collected from nine sampling sites, including 425 of yaks, 309 of Tibetan sheep, 266 of ticks. By morphological examination, PCR, and sequencing, we confirmed the species of all collected ticks. All tick samples, all yak and Tibetan sheep blood samples were detected based on SFG *Rickettsia ompA* and *sca4* gene. The results showed that all tick samples were identified to be *Haemaphysalis qinghaiensis*, and the positive rates of SFG *Rickettsia* were 5.9% (25/425), 0.3% (1/309), and 54.1% (144/266) in yaks, Tibetan sheep, and ticks, respectively. All positive samples were sequenced, and BLASTn analysis of the *ompA* gene sequences of SFG *Rickettsia* showed that all positive samples from animals and ticks had 99.04–100% identity with yak and horse isolates from Qinghai Province, China. BLASTn analysis of the *sca4* gene sequences of SFG *Rickettsia* showed that all positive samples had 97.60–98.72% identity with tick isolates from Ukraine. In addition, the phylogenetic analysis showed that all the SFG *Rickettsia ompA* and *sca4* sequences obtained from this study belong to the same clade as *Rickettsia raoultii* isolated from livestock and ticks from China and other countries. Molecularly, this study detected and

characterized SFG *Rickettsia* both in the tick vectors and animals, suggesting that the relationship between SFG *Rickettsia*, tick species and animal hosts should be explored to understand their interrelationships, which provide a theoretical basis for preventing control of this pathogen.

Keywords: Spotted Fever Group *Rickettsia*, *Haemaphysalis qinghaiensis*, yak, Tibetan sheep, Qinghai-Tibetan Plateau Area

INTRODUCTION

Rickettsia spp. (Rickettsiales: Rickettsiaceae) are obligate intracellular bacteria that causes rickettsioses, which is one of the oldest known vector-borne zoonoses diseases (1). The genus *Rickettsia* consists of four members: spotted fever group (SFG) rickettsiae, typhus group rickettsiae, the *Rickettsia bellii* group, and the *Rickettsia canadensis* group (2). At present, 26 SFG *Rickettsia* species had been reported, 16 of which were associated with human diseases (3, 4). In addition to the typical triad of fever, rash, and headache, the classic symptoms of new cases of *Rickettsia* varied by type, and in some cases, the typical symptoms might not appear or be ignored (4). Therefore, the clinical diagnosis of rickettsioses is a huge challenge for both physicians and veterinarians (3, 4).

SFG *Rickettsia* includes more than 20 different species, including pathogenic species that cause human and animal incidence, and nonpathogenic species (5). In Asia, a variety of SFG *Rickettsia* have been reported, and these rickettsiae are mostly distributed in border areas of various countries. In the Russian Far East, Kazakhstan, northern China, and Mongolia, *R. sibirica* and *R. raoultii* are widely distributed (4). In China, 11 SFG *Rickettsia* species have been detected in tick vectors and animals, some of which have been characterized as human pathogens (6). In previous reports, SFG *Rickettsia* was detected in a variety of ticks, including *Haemaphysalis qinghaiensis* in Yunnan Province and Harbin Province in China (5, 7, 8). However, there are few reports of the determination of SFG *Rickettsia* in *H. qinghaiensis* in the Qinghai-Tibetan Plateau Area (QTPA) (7, 9).

The QTPA is well known for the largest and highest plateau in the world, which is located in northwestern China and with a unique natural ecosystem (7). Yaks and Tibetan sheep are indigenous animals on the QTPA, which are the important sources of milk, meat, fur, skins, and dung in the area, contributing to the local economic development and the lives of herdsmen (10). Although SFG rickettsioses in livestock have been reported in this area, the information on these pathogens are limited (6, 11). *H. qinghaiensis* is a common and endemic three-host tick on the QTPA, which is easy to parasitize on Tibetan sheep, yaks, sheep, goats, and other domestic animals to transmit a variety of diseases (9). Therefore, the existence of *H. qinghaiensis* is a potential threat to the animal husbandry in the QTPA (12).

This study aimed to determine the existence and molecular characteristics of SFG *Rickettsia* from yaks, Tibetan sheep, and tick vectors in the QTPA, so as to further understand the

prevalence of SFG *Rickettsia* and clarify the interaction between ticks and animals.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

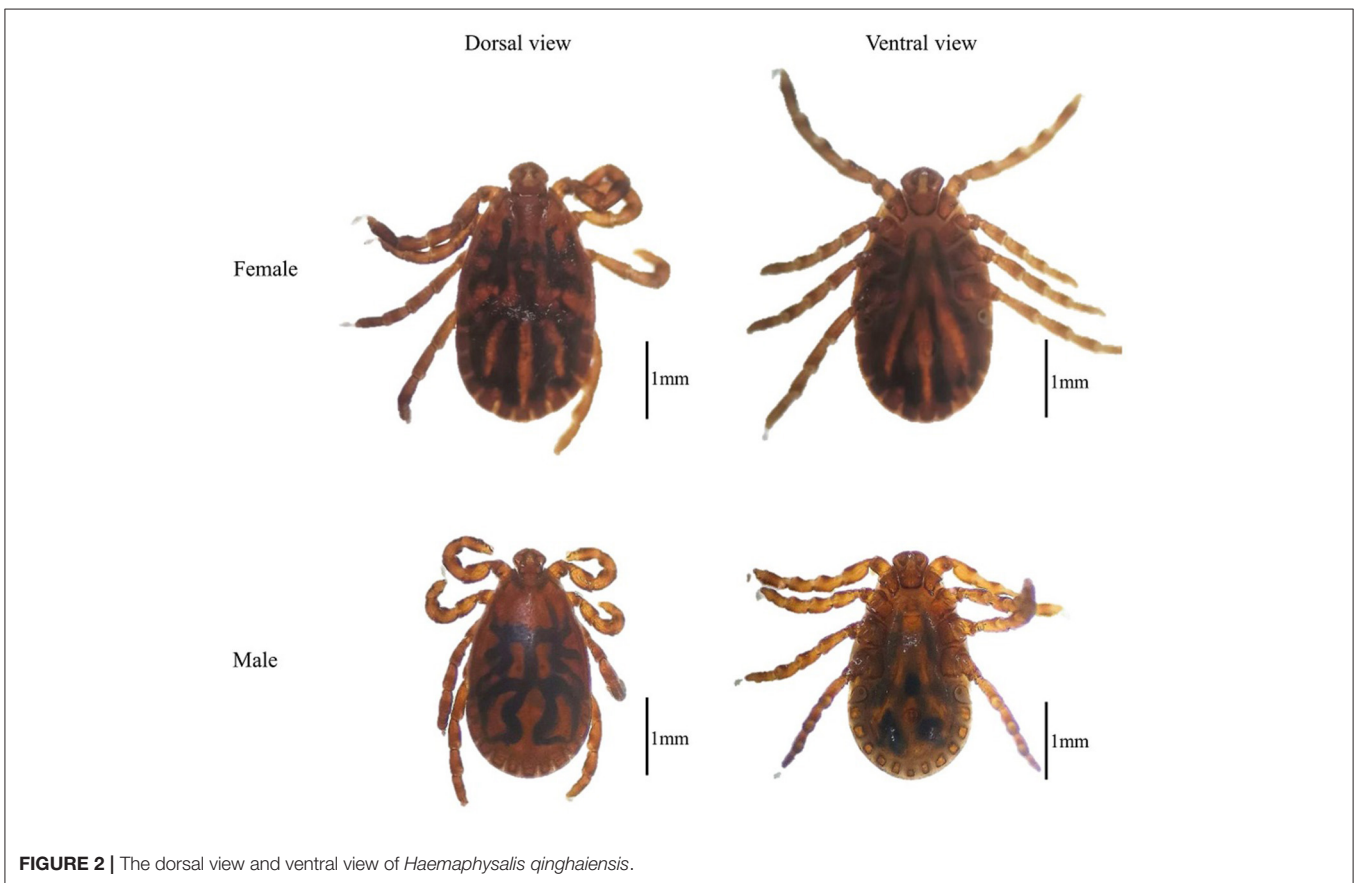
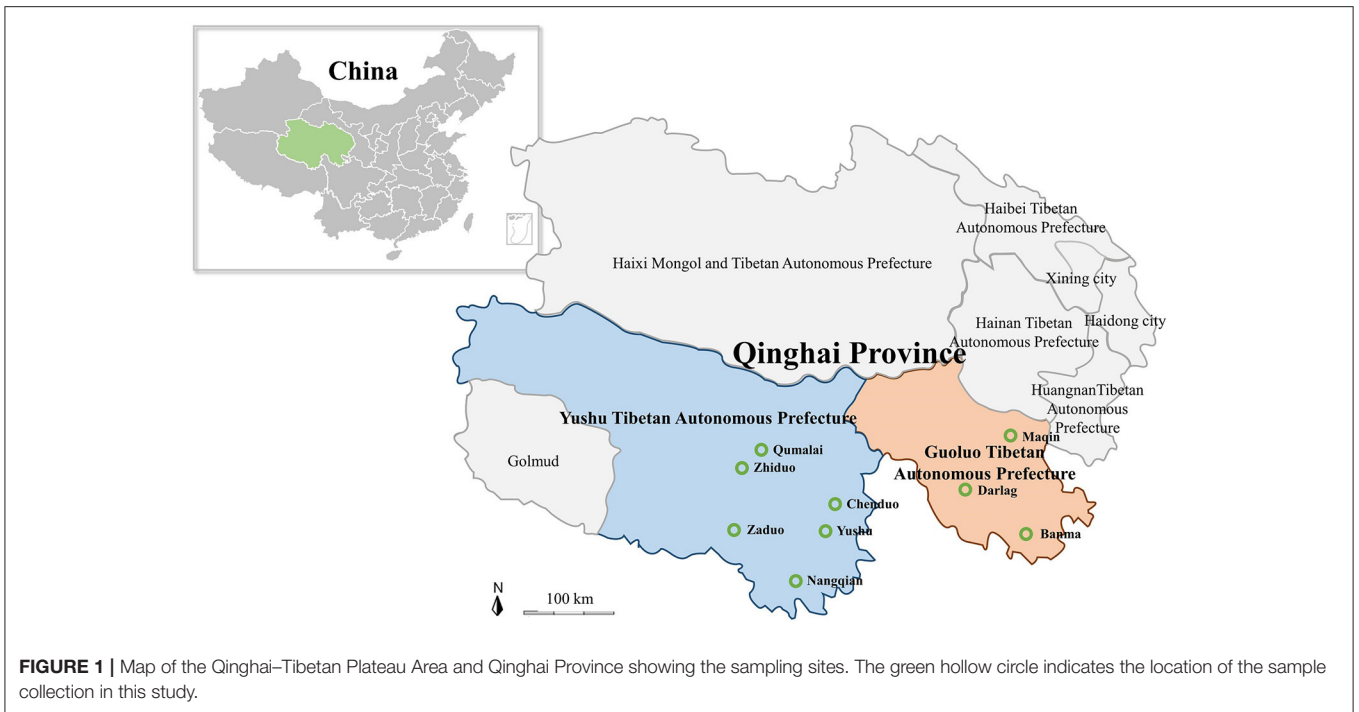
In the present study, all of 1,000 samples including yak and Tibetan sheep blood samples, and tick samples were collected from nine cities of Yushu Tibetan Autonomous Prefecture (Yushu) and Guoluo Tibetan Autonomous Prefecture (Guoluo) in Qinghai Province of QTPA (Figure 1). The 734 blood samples of yaks and Tibetan sheep were collected in tubes containing an EDTA anticoagulant, and genomic DNA was extracted using the MagPure Blood DNA KF Kit (Magen, China) according to the manual of the manufacturer. The 266 tick samples were collected, some of which were engorged ticks collected on the surface of yaks and Tibetan sheep, and the rest of ticks were collected by dragging the grasslands with a flannel cloth. DNA was extracted by using the MagPure Mollusc DNA KF Kit (Magen, China) and stored at -80°C until further use.

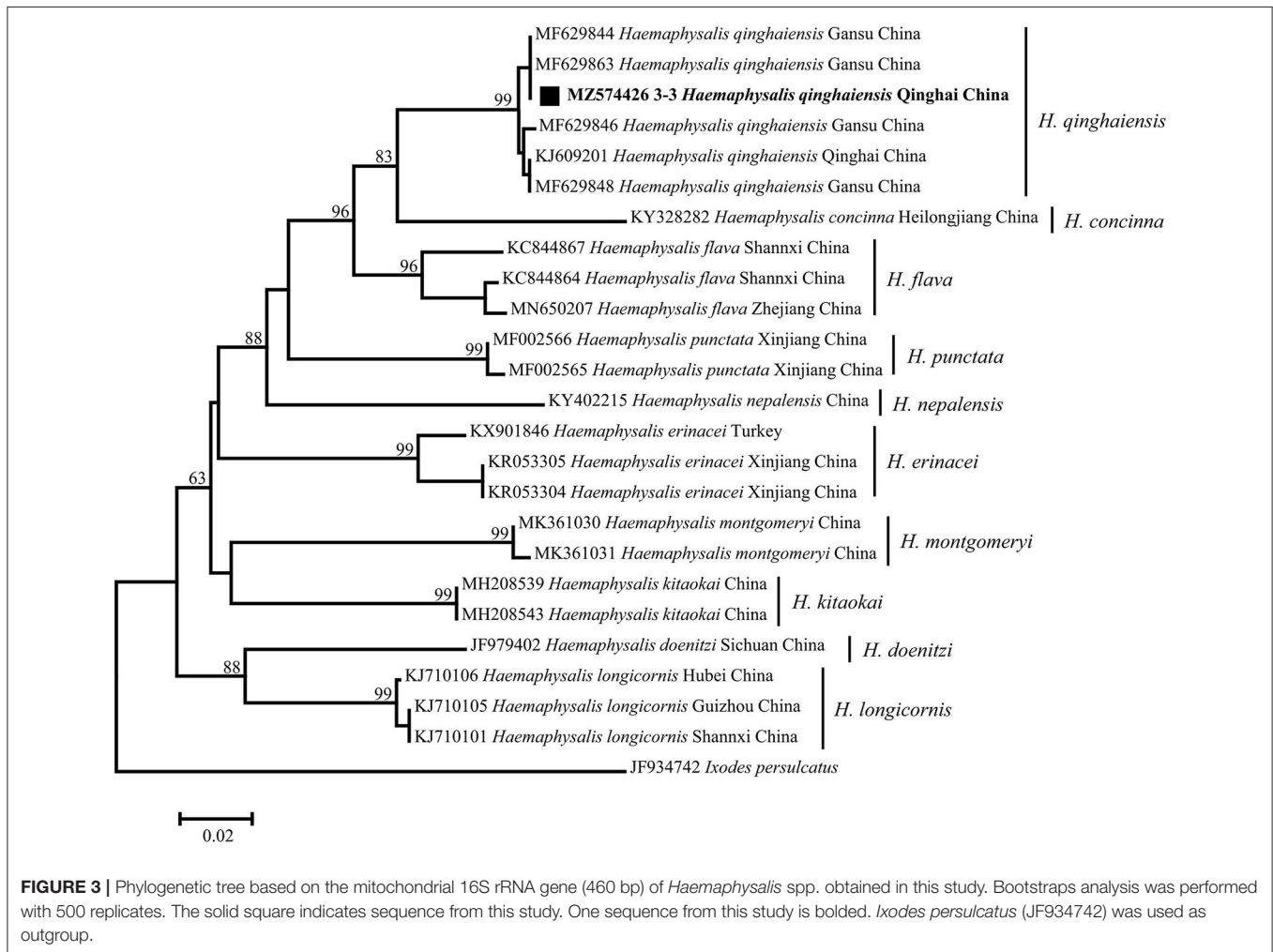
Identification of Ticks and Detection of Pathogens

Ticks were identified by the mitochondrial 16S rRNA gene (forward primer 5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3', reverse primer 5'-CCG GTC TGA ACT CAG ATC AAG T-3') and morphological characteristics (13, 14). Outer membrane protein A (*ompA*) gene and surface cell antigen 4 (*sca4*) gene were used to investigate the molecular characterization of the SFG *Rickettsia* by using genus-specific primers (forward primer 5'-GCT TTA TTC ACC ACC TCA AC-3' and reverse primer 5'-TRA TCA CCA CCG TAA GTA AAT-3'; D767f: 5'-CGA TGG TAG CAT TAA AAG CT-3' and D1390r: 5'-CTT GCT TTT CAG CAA TAT CAC-3') (15, 16). PCR reaction volume of 10 μl containing 3 μl of DNA template, 0.5 μl each of forward and reverse primer (100 μM), 0.1 μl of Taq polymerase (0.5 U; New England BioLab, USA), 0.2 μl of deoxyribonucleotide triphosphate (200 μM ; New England BioLab, USA), 1 μl of 10 \times ThermoPol Reaction Buffer (New England BioLab, USA), and double-distilled water to a volume of 10 μl . Positive yak DNA samples of *Rickettsia* spp. *ompA* gene from our previous study were used as positive controls, and double-distilled water was used as a negative control (11).

Sequencing and Phylogenetic Analyses

All PCR positive tick and pathogen samples were purified using EasyPure® Quick Gel Extraction Kit (TransGen, China) and cloned into *E. coli* DH5 α using the PMDTM 19-T Vector





Cloning Kit (TaKaRa, Japan). At least two positive clones from each positive sample were selected for sequencing in Sangon Biotech (Shanghai) Co., Ltd. Nucleotide sequence identities were determined by performing GenBank BLASTn analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic trees based on the obtained sequences were constructed using the neighbor-joining method, and genetic distance matrix among various *Rickettsia* clades in a phylogenetic tree were calculated using the maximum composite likelihood model in MEGA7.0 (17).

Statistical Analysis

The Chi-square test was used to evaluate the difference in prevalence between different parameters. Exposure variables included prefecture (Guoluo and Yushu), altitude (3,000–4,000 and 4,000–5,000 m), and animals (yak and Tibetan sheep). When the *p*-values were lower than 0.05, the result was considered to be of statistically significant difference (<https://www.mathsisfun.com/data/chi-square-calculator.html>).

RESULTS

Identification of Ticks

By morphological examination, all the tick samples were identified as *Haemaphysalis* (Figure 2) (14); then the

mitochondrial 16S rRNA gene target was used as an identification molecular marker, and 30 tick specimens were randomly selected for sequencing. By BLASTn analysis and phylogenetic analysis, the evidence demonstrated that there is an extensive homology between the *Haemaphysalis* species 16S rRNA gene sequence and the published sequence of *H. qinghaiensis* (MF629844) from Gansu Province, China, and grouped with the *H. qinghaiensis* clades (Figure 3 and Table 1).

Infection Rates of Spotted Fever Group *Rickettsia*

In the current study, the overall infection rate of SFG *Rickettsia* was 17% (170/1,000), including the infection rate of 51.35% (76/148) in animal-derived engorged ticks, 57.6% (68/118) in free ticks, 5.9% (25/425) in yaks, and 0.3% (1/309) in Tibetan sheep (Table 2).

Statistical analysis showed that for yaks, the infection rate of SFG *Rickettsia* in Guoluo was significantly higher than that of Yushu ($p = 0.0002$), and the infection rate at altitudes of 3,000–4,000 m was significantly higher than that at altitudes of 4,000–5,000 m ($p = 0.0005$) (Table 3). Moreover, altitude also was a significant impact factor on the total infection rate, such as the infection rate of SFG *Rickettsia* at an altitude

TABLE 1 | DNA sequences of spotted fever group (SFG) *Rickettsia* obtained in this study.

Tick and tick borne pathogens	Obtained sequences				The closest BLASTn match		
	Sample	Target gene	Accession number	Length (bp)	Identity (%)	Species	Accession number (host, country)
<i>Haemaphysalis</i> spp.	tick	16S rRNA	MZ574426	454	100	<i>H. qinghaiensis</i>	MF629844 China
<i>Rickettsia</i> spp.	Tick	<i>ompA</i>	MZ130274	209	100	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130275	207	100	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130276	209	100	<i>R. raoultii</i>	MN394800 yak China
	Tick		MZ130277	209	99.52	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130278	209	99.04	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130279	209	99.52	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130280	209	99.52	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130281	209	99.52	<i>R. raoultii</i>	MN394801 yak China
	Tick		MZ130282	208	99.04	<i>R. raoultii</i>	MN394800 yak China
	Tick		MZ130283	209	100	<i>R. raoultii</i>	MN394800 yak China
	Tick		MZ130284	209	100	<i>R. raoultii</i>	MN394800 yak China
	Tick		MZ130285	209	100	<i>R. raoultii</i>	MN394801 yak China
	Yak		MZ130267	209	100	<i>R. raoultii</i>	MK307883 horse China
	Yak		MZ130268	209	100	<i>R. raoultii</i>	MN394797 yak China
	Yak		MZ130269	209	100	<i>R. raoultii</i>	MN394797 yak China
	Yak		MZ130270	209	100	<i>R. raoultii</i>	MK307883 horse China
	Yak		MZ130271	209	99.52	<i>R. raoultii</i>	MN394798 yak China
Yak	MZ130272	209	99.52	<i>R. raoultii</i>	MN394797 yak China		
Tibetan sheep		MZ130273	209	100	<i>R. raoultii</i>	MK307883 horse China	
Tick	<i>sca4</i>	OL621221	624	98.72	<i>R. raoultii</i>	KP768191 tick Ukraine	
Tick		OL621222	624	98.56	<i>R. raoultii</i>	KP768191 tick Ukraine	
Tick		OL621223	624	98.40	<i>R. raoultii</i>	KP768191 tick Ukraine	
Tick		OL621224	624	97.60	<i>R. raoultii</i>	KP768191 tick Ukraine	
Tick		OL621225	624	97.92	<i>R. raoultii</i>	KP768191 tick Ukraine	

of 3,000–4,000 m was remarkably higher than that of 4,000–5,000 m ($p = 0.0183$) (Table 3). However, there was no significant difference in the infection rate of SFG *Rickettsia* from engorged *H. qinghaiensis* divided from yaks and Tibetan sheep, and also no significant difference in the infection rate of SFG *Rickettsia* of free *H. qinghaiensis* and engorged those in this study ($p > 0.05$) (Table 4).

Sequencing and Phylogenetic Analysis of Spotted Fever Group *Rickettsia ompA* Gene

In this study, a total of 19 SFG *Rickettsia ompA* gene sequences (MZ130267–MZ130285) were obtained, and the identity between each sequence was 94.2–100%, and the identity was 99.04–100% between MK307883, MN394797, MN394798, MN394801, and MN394800. The identity was 95.7–98.6% between the sequences of ticks and those of animals. Phylogenetic analysis of SFG *Rickettsia ompA* gene revealed that 19 sequences were in the same clade as *R. raoultii* isolated from horse, camel, *Haemaphysalis erinacei*, *Ixodes persulcatus*, and three *Dermacentor* species from China, Austria, and Turkey (Figure 4). Genetic distance among various *Rickettsia* clades was 1–21% and *R. raoultii* with 8–13% genetic distance with other rickettsiae (Table 5).

Sequencing and Phylogenetic Analysis of Spotted Fever Group *Rickettsia sca4* Gene

All of five SFG *Rickettsia sca4* gene sequences (OL621221–OL621225) were obtained, and the identity between each sequences was 98.6–99.8%, and the identity was 97.60–98.72% in KP768191. Phylogenetic analysis of SFG *Rickettsia sca4* gene revealed that five sequences were in the same clade as *R. raoultii* isolated from *Dermacentor reticulatus* from Ukraine (Figure 5). Genetic distance among various *Rickettsia* clades was 1–42%, *R. raoultii* with 2–25% genetic distance with other rickettsiae (Table 6).

DISCUSSION

Haemaphysalis qinghaiensis was first discovered in Huangyuan County, Qinghai Province in 1980, and was subsequently discovered in Gansu, Sichuan, Ningxia, Yunnan, Tibet provinces of China (14, 18). There was no record abroad. In nature, a complete life history of *H. qinghaiensis* is generally 3 years (14). It was the dominant tick species in Qinghai Province of the QTPA, China, and it is mainly parasitic on various domestic animals, and as the vector of *Theileria* sp. and *Babesia* sp. (12, 14). Therefore, *H. qinghaiensis*

TABLE 2 | The positive samples of spotted fever group (SFG) *Rickettsia* in ticks and animals in this study.

Prefectures	Sampling sites	Altitude (m)	Number of positive samples (Infection rate %)				
			Tick-E*	Tick-F*	Yak	Tibetan sheep	Total
Yushu	Yushu	4,117–4,317	6/15 (40.0)	20/38 (52.6)	0/53	0	26/106 (24.5)
	Zhiduo	4,171.4	17/37 (46.0)	0	0/30	0	17/67 (25.4)
	Zaduo	4,290	0	0	0/33	0	0/33
	Chenduo	4,000	0	10/17 (58.8)	0/29	0/25	10/71 (14.1)
	Nangqian	3,640–3,920	0	13/18 (72.2)	0/48	0/51	13/117 (11.1)
	Qumalai	4,279	0	0	3/22 (13.6)	1/50 (2.0)	4/72 (1.4)
	Total		23/52 (44.2)	43/73 (58.9)	3/215 (1.4)	1/126 (0.8)	70/466 (15.0)
Guoluo	Banma	3,623–3,877	0	15/25 (60.0)	10/78 (12.8)	0	25/103 (24.3)
	Darlag	4,130	2/9 (22.2)	0	0/35	0/51	2/95 (2.1)
	Maqin	3,859	51/87 (58.6)	10/20 (50.0)	12/97 (12.4)	0/132	73/336 (21.7)
	Total		53/96 (55.2)	25/45 (55.6)	22/210 (10.5)	0/183	100/534 (18.7)
Total			76/148 (51.35)	68/118 (57.6)	25/425 (5.9)	1/309 (0.3)	170/1,000 (17.0)

*E, engorged ticks collected from yaks or Tibetan sheep in this study; F, free ticks on the grass.

TABLE 3 | The patterns of infection rates of spotted fever group (SFG) *Rickettsia* in ticks, yaks, and Tibetan sheep grouped by prefecture and altitude of the sampling sites.

Parameter	Number of positive samples (infection rate %)				
	Tick-E*	Tick-F*	Yak	Tibetan sheep	Total
Prefecture					
Yushu	23/52 (44.2)	43/73 (58.9)	3/215 (1.4)	1/126 (0.8)	70/466 (15.0)
Guoluo	53/96 (55.2)	25/45 (55.6)	22/210 (10.5)	0/183 (0)	100/534 (18.7)
p-Value	0.4644	0.8526	0.0002	0.2292	0.1895
Altitude (m)					
3,000–4,000	51/87 (58.6)	38/63 (60.3)	22/223 (9.9)	0/183 (0)	111/556 (20.0)
4,000–5,000	25/61 (41.0)	30/55 (54.5)	3/202 (1.5)	1/126 (0.8)	59/444 (13.3)
p-value	0.2253	0.7424	0.0005	0.2292	0.0183

*E, engorged-ticks collected from yaks or Tibetan sheep in this study; F, free ticks on the grass.

and its transmitted diseases were the main economic burden of animal husbandry in Qinghai Province in the QTPA (12).

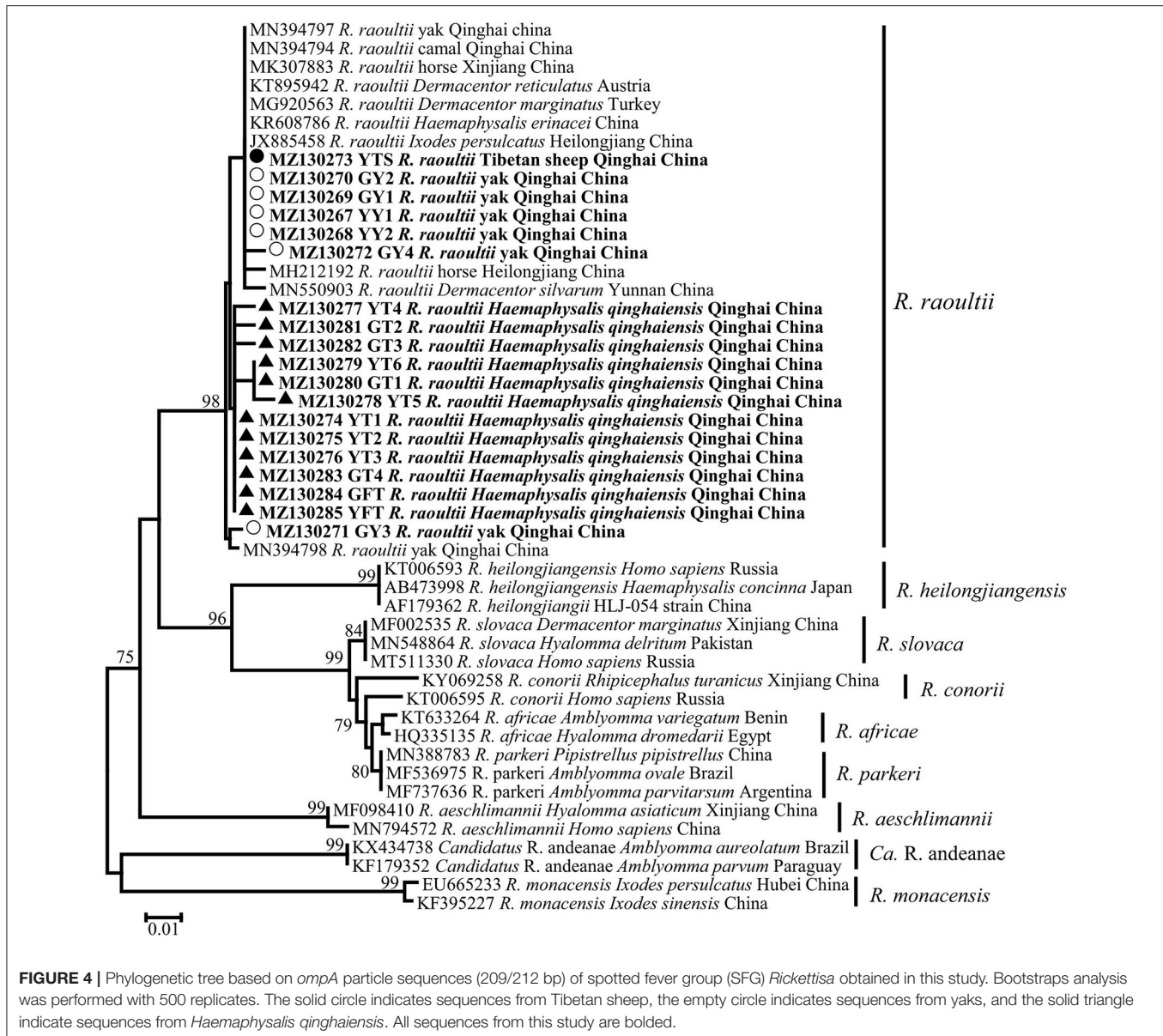
In the past 30 years, the scope and importance of pathogenic rickettsiae associated with ticks had increased dramatically (19). Most of the pathogenic *Rickettsia* species discovered had been found in ticks, and ticks were considered to be the most important vector and reservoir host of *Rickettsia* in almost every region of the world (4, 20, 21). Later, they were discovered to be related to disease symptoms in humans, such as headache, arthralgias, multiple eschars, and meningoencephalitis (22, 23). Although ticks were parasitic on humans appearing to be opportunistic, the risk of *Rickettsia* infection was greatly increased among herders and livestock living on the Tibetan Plateau, where ticks are widely distributed.

TABLE 4 | The infection rate of spotted fever group (SFG) *Rickettsia* in engorged ticks.

Prefectures	Number of positive engorged tick samples (infection rate %)		
	Yak	Tibetan sheep	p-Value
Yushu	17/37 (46.0)	6/15 (40.0)	0.8061
Guoluo	3/8 (37.5)	50/88 (56.8)	0.5503
Total	20/45 (44.4)	56/103 (54.4)	0.5231

R. raoultii is a bacterium that was first isolated in 1999 from *D. nuttalli* and *Rhipicephalus pumilio* and was described as a novel species in 2008 (24, 25). In China, the first reported case of *R. raoultii* in 2012 was from *D. silvarum* in Xinjiang Province (26). In a previous report of Wen et al., *R. raoultii* was detected in at least 13 species of ticks belonging to six genera, including *Dermacentor*, *Ixodes*, *Rhipicephalus*, *Haemaphysalis*, *Amblyomma*, and *Hyalomma* (27). Moreover, *R. raoultii* was detected in animals, such as yaks, Bactrian camel (*Camelus bactrianus*), and red fox (*Vulpes vulpes*) (11, 28). Importantly, *R. raoultii* was considered to be a human pathogen that had been shown to cause Tick-borne lymphadenopathy (TIBOLA) and *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL) (29), and it should be emphasized that the cases had also been reported in China (30, 31).

ompA is considered to be a good candidate gene for phylogenetic analysis of most SFG representatives, with a higher degree of interspecies variation than 16S rRNA and citrate synthase-encoding gene (*gltA*), and *ompA* is present in almost all SFG members (32). *sca4* also has been used to rapidly and reliably differentiate members of the genus *Rickettsia* (16). Thus, *ompA* and *sca4* gene fragments were used to specifically detect rickettsial infection in ticks in many studies, such as Ishikura et al. (33), Orkun et al. (34), Taylor et al. (35), Jiang et al. (36), Kooshki et al. (37) and Nadim et al. (38). Therefore, in the



current study, *ompA* and *sca4* were selected as the target genes to detect SFG *Rickettsia* in yak, Tibetan sheep blood samples, and tick samples. This study results showed that SFG *Rickettsia* infection was detected in both tick and animal blood samples. By sequencing and BLASTn analysis, the evidence demonstrated that there were 99.04–100% sequence identities between obtained SFG *Rickettsia ompA* sequences (MZ130267–MZ130285) and *R. raoultii* (MK307883, MN394797, MN394798, MN394801, and MN394800) isolated from China. Meanwhile, BLASTn analysis of *sca4* sequences showed 97.60–98.72% similarity with *R. raoultii* (KP768191) isolated from *D. reticulatus* from Ukraine. Furthermore, phylogenetic analysis showed that the current study obtained DNA sequences that formed an independent clade with *R. raoultii* from China, Austria, Turkey, Ukraine, and Russia, with a high bootstrap value. The genetic distance matrix

based on *Rickettsia ompA* and *sca4* genes showed that the genetic distances between *R. raoultii* and other rickettsiae were 8–13% and 2–25%, respectively (Tables 5, 6). In previous studies, this pathogen was also reported in various provinces such as Jilin (39), Heilongjiang (30), Gansu (40), Shaanxi (40), Xinjiang (26), and Yunnan (5), which suggests that the geographic distribution of *R. raoultii* covered most of China.

Molecular biology detection revealed that bacteria of genus *Rickettsia* were distributed all over the world, which was related to the geographical distribution of their vectors (4). Ticks, as the most important vectors of rickettsiae, were distributed throughout the world; the risk of rickettsioses in a given region might depend on the presence and biotopes of specific species of ticks (41). Among Asian countries, *R. sibirica* was endemic in most of the areas of Russia, China, Mongolia, and

Kazakhstan, and was mainly transmitted by the *Dermacentor* tick (3). In addition, it was reported in Russia and China that *R. heilongjiangensis* isolated from *H. concinna* and *D. silvarum*, and the cases caused by this *Rickettsia* species, had also been reported in Japan (1, 42). *R. japonica* was also found in Japan and was transmitted by a variety of *Haemaphysalis* ticks, other

Ixodes and *Dermacentor* ticks (1). *Rickettsia* strains closely related to *R. japonica* were detected in ticks in South Korea and northern Thailand (43, 44). In Turkey, researchers detected different SFG *Rickettsia* in different ticks; *R. aeschlimanii* was detected in *H. marginatum*, *R. raoultii* and *R. slovaca* were detected in *D. marginatus*, and *R. hoogstraalii* in *H. parva* (45). In India, *R. conorii* subsp. *indica* was widespread, and the cases were also reported in Laos and Sri Lanka (1). Ticks are the most important external parasites of livestock and wildlife in Iran. A total of 46 species had been reported (46), in which *Argas persicus* was the important ectoparasite of poultry in Iran. In previous studies, *R.*

TABLE 5 | Genetic distance matrix among various *Rickettsia* species in a phylogenetic tree based on the *ompA* gene in this study.

Rickettsial species	Evolutionary divergence over sequence pairs between rickettsial species (%)								
	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. <i>R. raoultii</i>									
2. <i>R. slovaca</i>	8								
3. <i>R. aeschlimanii</i>	9	13							
4. <i>R. parkeri</i>	9	1	14						
5. <i>R. conorii</i>	10	1	14	1					
6. <i>Candidatus R. andeanae</i>	12	14	16	14	15				
7. <i>R. africae</i>	9	1	14	1	2	15			
8. <i>R. heilongjiangensis</i>	9	8	14	8	10	15	9		
9. <i>R. monacensis</i>	13	19	15	20	20	16	21	18	

TABLE 6 | Genetic distance matrix among various *Rickettsia* species in a phylogenetic tree based on *sca4* gene in this study.

Rickettsial species	Evolutionary divergence over sequence pairs between rickettsial species (%)					
	1.	2.	3.	4.	5.	6.
1. <i>R. raoultii</i>						
2. <i>R. heilongjiangensis</i>	2					
3. <i>R. parkeri</i>	3	3				
4. <i>R. monacensis</i>	24	10	6			
5. <i>R. slovaca</i>	2	1	1	5		
6. <i>R. prowazekii</i>	25	28	26	42	23	

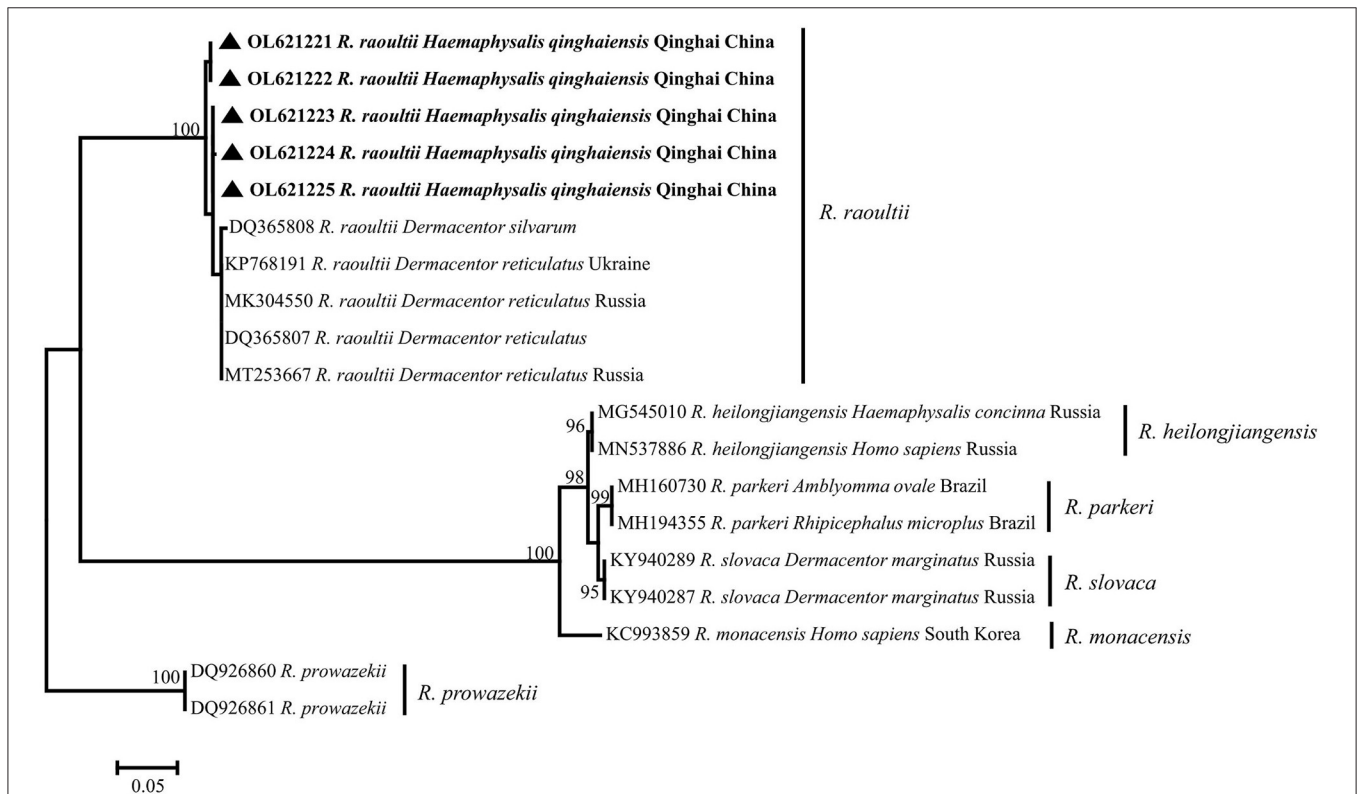


FIGURE 5 | Phylogenetic tree based on *sca4* particle sequences (624 bp) of spotted fever group (SFG) *Rickettsia* obtained in this study. Bootstraps analysis was performed with 500 replicates. The solid triangle indicates sequences from *Haemaphysalis qinghaiensis*. All sequences from this study are bolded.

Hoogstraalii (37) and SFG *Rickettsia* (47) had been detected in *Argas persicus*. In addition, the epidemiological investigation on SFG *Rickettsia* of *D. marginatus* Sulzer, *H. sulcata*, *H. inermis*, and *Hyalomma asiaticum* in Iran was reported (38, 47).

In the current study, the infection rates of SFG *Rickettsia* in free *H. qinghaiensis*, engorged *H. qinghaiensis*, yaks, and Tibetan sheep were 57.6%, 51.35%, 5.9%, and 0.3%, respectively. Studies had reported the detection of *R. raoultii* in yaks, camels, and several ticks in Qinghai Province (7, 11). Combined with the experimental results of this study, it was suggested that *H. qinghaiensis* might be the host of this pathogen, but because the infection rate of this pathogen between animals and ticks was remarkably different, therefore, whether the pathogen could be transmitted by *H. qinghaiensis* needed further study. Meanwhile, a phylogenetic tree showed that the *ompA* sequences of SFG *Rickettsia* isolated from *H. qinghaiensis* in Qinghai belonged to the same clade as the reported one, while the *ompA* gene sequences of SFG *Rickettsia* isolated from animals (except MZ130271) were in same clade as those of other tick species (including *Dermacentor* and *Ixodes*). In addition, Han et al. also detected this pathogen in several *Dermacentor* ticks in Qinghai Province (7). According to a previous report (48) that *R. raoultii* was closely associated with *Dermacentor* spp., we suspected that this pathogen isolated from animals in this study was not mainly transmitted by *H. qinghaiensis*. The high infection rate of this pathogen in *H. qinghaiensis* suggested that *H. qinghaiensis* might be the reservoir of *R. raoultii*.

In the current study, the survey results showed that the infection rate of SFG *Rickettsia* in yaks was affected by region and altitude ($p = 0.0002$ and $p = 0.0005$), and the total infection rates were affected by altitude ($p = 0.018$). However, the current infection rates of SFG *Rickettsia* showed no significant difference between yaks and Tibetan sheep, which implied that the infection of SFG *Rickettsia* might not be related to the host species in detected areas. So further research was needed to confirm this suspicion.

In conclusion, this study revealed the existence of SFG *Rickettsia* in tick vectors, yaks, and Tibetan sheep in QTPA, China. The phylogenetic analysis showed that all sequences obtained in the current study were closely related to the *R. raoultii* isolated from different tick species and domestic

animals. Although the case of human infection by *R. raoultii* was not reported in the sampling regions, high infection rates should be paid more attention. Importantly, the relationship between SFG *Rickettsia*, tick species, and animal hosts should be further explored to understand their interrelationships.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The study was conducted in compliance with the ethical policies of the journal and the rules of the Ethics Committee of Qinghai University.

AUTHOR CONTRIBUTIONS

YL, Y-CH, J-XL, Y-LS, H-XH, J-SC, Y-HG, YW, PM, Y-PW, R-SL, W-KC, Z-HC, and JL designed the experiments and sampling. Y-CH, PM, Y-PW, R-SL, W-KC, and Z-HC performed the experiments. Y-CH and J-XL analyzed the data. Y-CH wrote the original manuscript. T-SQ, J-FY, Q-XZ, J-YC, Q-BZ, G-WH, MK, and YL reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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