

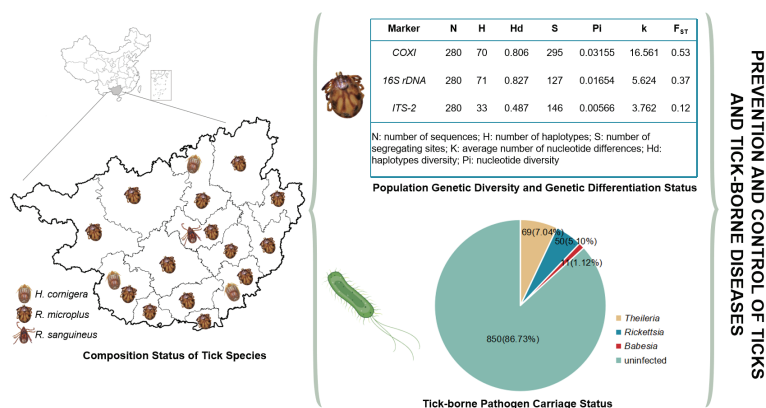
ORIGINAL ARTICLE



Species Composition, Genetic Structure, and Pathogen Prevalence in Tick Populations in Guangxi, China

Graphical abstract

Species composition, Genetic Structure and Pathogen Prevalence in Tick Populations of Guangxi, China



Authors

Na Jiang, Haoqiang Tian, Chunfu Li, Rui Ma, Mengyun Liu, Shurong Wang, Qingan Zhou, Xiankai Wei, Juanmei Mo, Zimei Chen, Zhaoan Sheng, Lei Duan, Lu He, Peng Song, Jiahui Sun, Jian Li, Wei Hu and Xinyu Feng

Corresponding authors

leejianshin@163.com (J. Li);
huw@imu.edu.cn (W. Hu);
fengxinyu2013@163.com (X. Feng)

Highlights:

- The study identified three tick species in Guangxi. *Rhipicephalus microplus* was the dominant tick species in Guangxi, comprising 92.04% of samples and found in all surveyed cities.
- Genetic diversity was highest for 16S rDNA and COXI genes. Significant differentiation was observed between populations in Guilin, Hechi, and other areas.
- The overall pathogen infection rate was 13.27%, with *Rickettsia*, *Babesia*, and *Theileria* identified. Co-infections were present, but *Borrelia* and *Anaplasma* were not detected.

Brief Statement

This study highlights the substantial presence and genetic diversity of *Rhipicephalus microplus* ticks in Guangxi, China, including *Rickettsia*, *Babesia*, and *Theileria*, and emphasizes their roles as vectors for tick-borne diseases (TBDs). The detection of these pathogens, alongside instances of co-infection, underscores the persistent risk of TBDs in the region. The comprehensive epidemiological data provided are crucial for formulating targeted interventions to prevent and control TBDs in Guangxi and other regions.

Species Composition, Genetic Structure, and Pathogen Prevalence in Tick Populations in Guangxi, China

Na Jiang¹, Haoqiang Tian¹, Chunfu Li¹, Rui Ma¹, Mengyun Liu¹, Shurong Wang¹, Qingan Zhou², Xiankai Wei², Juanmei Mo³, Zimei Chen⁴, Zhaoan Sheng^{1,5}, Lei Duan^{6,7}, Lu He^{8,9}, Peng Song⁶, Jiahui Sun⁶, Jian Li^{1,10,*}, Wei Hu^{1,8,11,*} and Xinyu Feng^{8,9,*}

Abstract

Background: Ticks are important vectors for many pathogens affecting humans and animals. This study was aimed at investigating the species composition, genetic diversity, and potential transmission risks of tick-borne pathogens in the Guangxi Zhuang Autonomous Region, China.

Methods: A total of 980 ticks were collected from 14 prefecture-level cities in Guangxi between March and July 2021. Ticks were identified morphologically and genetically, and the population diversity of *Rhipicephalus microplus* was analyzed according to 16S rDNA, *COXI*, and *ITS-2* gene sequences. Population genetics was assessed through F_{ST} , AMOVA, and phylogenetic analyses. PCR was used to detect five common tick-borne pathogens: *Rickettsia*, *Babesia*, *Theileria*, *Borrelia burgdorferi*, and *Anaplasma phagocytophilum*.

Results: *Rhipicephalus microplus* was the dominant tick species, and was observed in 92.04% of all samples. Genetic diversity analysis revealed higher haplotype diversity for the 16S rDNA and *COXI* genes than the *ITS-2* gene; these findings suggested a faster evolutionary rate for the first two markers. Population genetic differentiation was observed between *Rhipicephalus microplus* populations in Guilin, Hechi, and other regions, probably because of geographic isolation. Pathogen analysis revealed a 13.27% overall infection rate, and identified three *Rickettsia*, two *Babesia*, and three *Theileria* species. Co-infection with *Theileria* and *Rickettsia* was also detected. Notably, neither *Borrelia* nor *Anaplasma* was found in the tick samples. Phylogenetic analyses confirmed the distinctiveness of the *Rickettsia* and *Piroplasma* groups.

Conclusions: This study provides a comprehensive assessment of tick species diversity and tick-borne pathogens in Guangxi, highlighting the potential health risks posed by *Rhipicephalus microplus*. The findings emphasize the need for expanded tick surveillance, enhanced pathogen detection methods, and integrated management strategies to mitigate the spread of tick-borne diseases. Future research should include the collection of free questing ticks and metagenomic approaches to provide a more comprehensive understanding of pathogen risk in the region.

Key words: Guangxi, *Rhipicephalus microplus*, Genetic diversity, Tick-borne pathogens

*Corresponding authors

E-mail: leejianshin@163.com (JL); huw@imu.edu.cn (WH); fengxinyu2013@163.com (XF)

¹College of Life Sciences, Inner Mongolia University, Hohhot, China

²Guangxi Center for Animal Disease Control and Prevention, Nanning, Guangxi, China

³Department of Oncology, Guangxi International Zhuang Medicine Hospital, Guangxi University of Chinese Medicine, Nanning, Guangxi, China

⁴Guangxi Nanning City Wildlife Protection Station, Nanning, Guangxi, China

⁵Department of Pathogenic Biology, Jining Medical University, Jining, China

⁶National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Chinese Center for Tropical Diseases Research); NHC Key Laboratory of Parasite and Vector Biology; WHO Collaborating Centre for Tropical Diseases; National Center for International Research on Tropical Diseases, Shanghai, China

⁷School of Life Science, Fudan University, Shanghai 200438, People's Republic of China

⁸School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China

⁹One Health Center, Shanghai Jiao Tong University-The University of Edinburgh, Shanghai, 20025, China

¹⁰Basic Medical College, Guangxi University of Chinese Medicine, Nanning, Guangxi, China

¹¹Department of Infectious Diseases, Huashan Hospital, State Key Laboratory of Genetic Engineering, Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Ministry of Education Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China

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INTRODUCTION

Ticks are specialized blood-feeding ectoparasites that rank among the foremost vectors of diseases affecting humans and domestic animals worldwide. Tick-borne diseases (TBDs) pose a substantial health burden, affecting millions of people each year [1–4]. In China, more than 3 million individuals are bitten by ticks annually in provinces such as Heilongjiang, Jilin, Liaoning, and Inner Mongolia, and approximately 30,000 cases of Lyme disease are reported [5]. Tick-borne encephalitis, Crimean-Congo hemorrhagic fever, and Q-fever are also prevalent, particularly in northern and western China, and sporadic outbreaks have been reported in recent years. Emerging diseases such as human monocytic ehrlichiosis and human granulocytic anaplasmosis, increasingly reported since the late 1990s, have led to growing public health concerns. Severe fever with thrombocytopenia syndrome (SFTS), caused by a novel bunyavirus, has rapidly spread since 2007, and more than 600 cases were reported by 2011. Additionally, diseases such as babesiosis, affecting both humans and animals, have shown expanded geographical spread, particularly in eastern and southern regions of China [5,6]. The increasing prevalence of TBDs underscores the need for comprehensive studies to better understand the epidemiology and transmission dynamics of these pathogens.

China hosts a diverse array of tick species, including 130 identified species across two families and nine genera [7], predominantly within the Ixodidae family. Many of these ticks serve as vectors for a wide range of pathogens, including bacteria, viruses, and protozoa [8]. Studies have reported high infection rates with pathogens such as *Rickettsia* spp., *Anaplasma* spp., *Borrelia* spp., and *Babesia* spp. in both tick vectors and their animal hosts [5–8]. The expanding geographic range of infected ticks with changing climatic conditions further emphasizes the importance of understanding tick species distribution and pathogen prevalence to achieve effective disease surveillance and control strategies.

Guangxi, located in China's tropical and subtropical zones, provides an ideal habitat for tick proliferation, because of its hilly terrain and lush vegetation. An early survey by Liao et al. documented 11 tick species across six genera in Guangxi in 1993 [9]. Subsequent studies have expanded on these findings, revealing a rich diversity of tick species in the region, dominated primarily by ticks of the genus *Rhipicephalus* in the Ixodidae family [10–12]. The spectrum of tick-borne pathogens identified in ticks from Guangxi includes the species *Rickettsia*, *Babesia*, *Anaplasma*, and viruses responsible for SFTS [13–18].

Despite advances in detection methods and increased attention to TBDs, the ecological distribution, genetic characteristics, and pathogen carriage rates of ticks in Guangxi have not been comprehensively explored. This knowledge gap is particularly concerning, given the region's rapid economic development, environmental changes, and intensification of the livestock trade, which may influence tick populations and disease transmission

dynamics. This study was aimed at elucidating the species distribution and genetic diversity of ticks in Guangxi through morphological and molecular analyses, focusing on mitochondrial 16S ribosomal DNA (16S rDNA), cytochrome c oxidase subunit I (COXI), and internal transcribed spacer 2 (ITS-2) gene sequences. Additionally, we sought to assess the genetic differentiation and historical dynamics within *Rhipicephalus microplus* (*R. microplus*) populations, alongside the detection and phylogenetic analysis of major tick-borne pathogens, including *Rickettsia*, *Babesia*, *Theileria*, *Borrelia burgdorferi* (*B. burgdorferi*), and *Anaplasma phagocytophilum* (*A. phagocytophilum*). The findings are expected to provide valuable insights into the potential risks of TBDs in Guangxi, and to contribute to the development of informed strategies for disease control and prevention in public health and animal husbandry.

METHODS

Sample collection

Between March and July 2021, 980 tick samples were collected from 14 prefecture-level cities in the Guangxi Zhuang Autonomous Region, China: Liuzhou, Hechi, Chongzuo, Guigang, Yulin, Laibin, Beihai, Baise, Qinzhou, Fangchenggang, Wuzhou, Hezhou, Nanning, and Guilin (Fig 1). All ticks were parasitic on host animals at the time of collection. They were manually removed with tweezers and placed into collection tubes with perforated caps for ventilation. The samples were rapidly transported to the laboratory, where each was cataloged in a database, identified, and processed for later analysis.

Tick processing and genomic DNA preparation

Before morphological identification, ticks were cleaned to remove soil, feces, and animal hair, to ensure sample integrity. Each tick was soaked in distilled water for 1 min, washed three times in 75% ethanol to eliminate surface bacteria, and finally rinsed with distilled water. After cleaning, tick species were identified according to their morphological characteristics. The ticks were placed on ice, and their legs were extended with tweezers and observed under a stereoscopic microscope (SMZ18/FL, Nikon, Japan). Identification was guided by descriptions from *Medical Arthropodology* [19].

After morphological sorting, ticks were placed individually in 1.5 mL centrifuge tubes, grouped by species, collection site, and host for genomic DNA extraction. Each sample was then mixed with 50 μ L phosphate-buffered saline, and tick tissues were disrupted to form suspensions with a high-throughput tissue homogenizer (FAST-24, Thermo Fisher, USA). After centrifugation, DNA was extracted from the supernatants with a DNeasy Blood and Tissue Kit (QIAGEN, Germany).

Molecular identification of ticks

To confirm the tick species, we performed PCR amplification of the DNA extracted from tick tissues, targeting

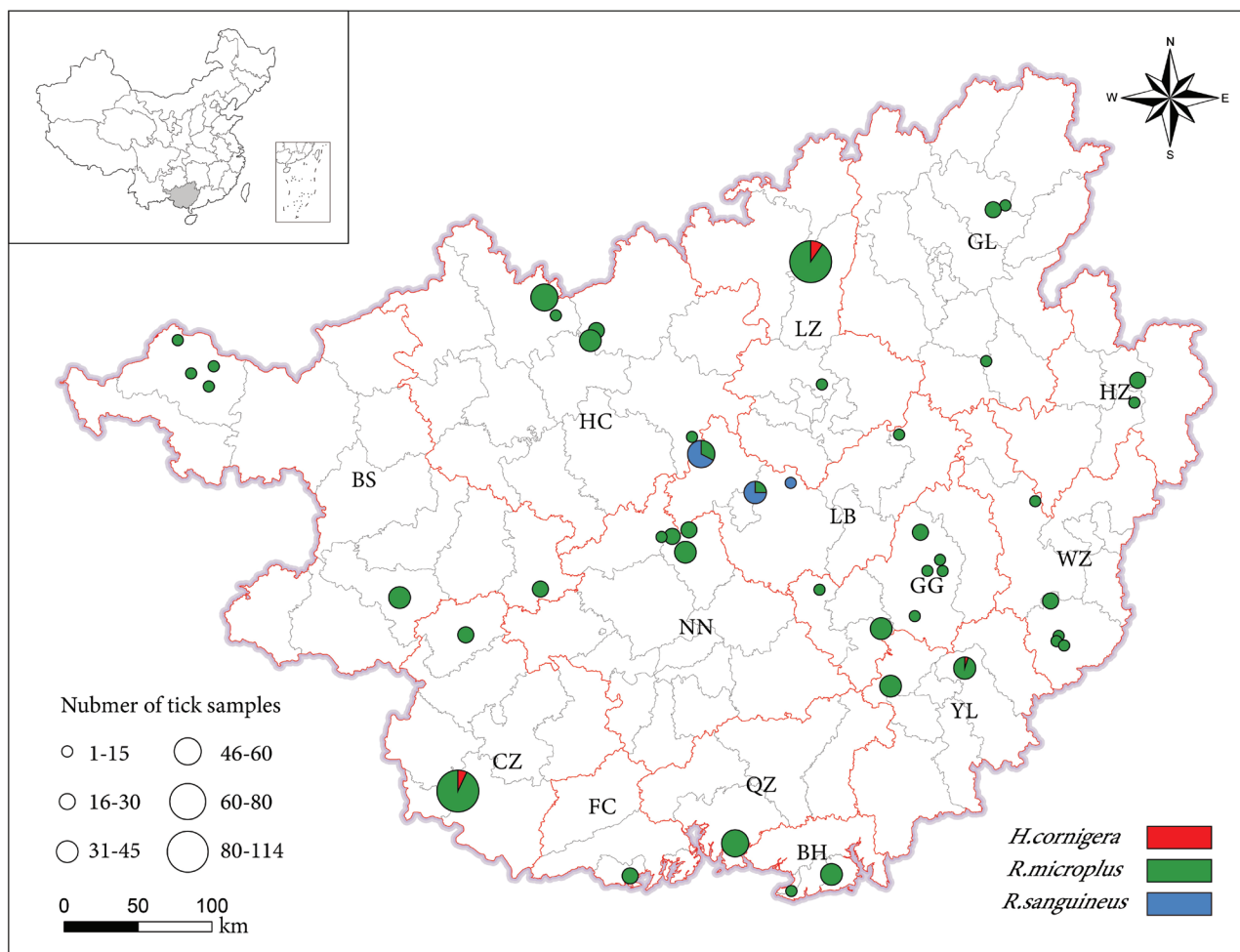


FIGURE 1 | Geographic distribution and species composition of tick collections. The figure shows the geographic locations of the 14 prefecture-level cities in the Guangxi Zhuang Autonomous Region in this study: BH (Beihai), BS (Baize), CZ (Chongzuo), FC (Fangchenggang), GG (Guigang), GL (Guilin), HC (Hechi), HZ (Hezhou), LB (Laibin), LZ (Liuzhou), NN (Nanning), QZ (Qinzhou), WZ (Wuzhou), and YL (Yulin). Circle size indicates the range of sample sizes at that sampling site. Colors represent different tick species (green for *R. microplus*, red for *H. cornigera*, and blue for *R. sanguineus*).

the 16S rDNA, *COXI*, and *ITS-2* genes [20–22]. Each 25 μ L PCR reaction contained 1.5 μ L DNA sample, 0.5 μ L each of forward and reverse primers, 12.5 μ L 2 $\times$ EasyTaq PCR SuperMix (Transgen, China), and 10 μ L ddH₂O. Details on PCR conditions and primers are provided in S1 Table. PCR products were analyzed with 2% agarose gel electrophoresis, and bands of the expected size were confirmed by Sanger sequencing. The DNA sequence of the unknown species was compared against a database of representative sequences from known species, and the identity of the unknown species was confirmed through evaluation of the alignment results, including similarity, identities, score, and gap penalties. The BLASTn parameters used for tick species identification in this study were as follows: E value 0.00–0.01, query coverage 96%–100%, and percentage identity 98%–100%.

Population genetic analysis

Genetic diversity analysis

To assess the genetic diversity of *R. microplus*, we calculated diversity indices with three genetic markers: 16S

rDNA, *COXI*, and *ITS-2* genes. The haplotype diversity (*H_d*) and nucleotide diversity (*P_i*) were computed for each gene in DnaSP v5.1 software. These indices provided insights into the genetic variability within the populations across geographic regions.

Population demographic history

To infer the population demographic history, we tested for signals of population expansion or bottleneck events. Neutrality tests were conducted with Tajima's *D* and Fu's *F_s* metrics in DnaSP v5.1. Mismatch distribution analysis was performed to detect demographic changes, including assessment of whether the populations had undergone recent expansions or decreases in size.

Population genetic differentiation

Population genetic differentiation was evaluated by calculation of the *F_{ST}* and *G_{ST}* coefficients in Arlequin v3.0 software. These indices quantified the degree of genetic differentiation between populations. Additionally, analysis of molecular variance (AMOVA) was conducted in

Arlequin v3.0 to assess the hierarchical partitioning of genetic variation, both within and between populations, and to determine the sources of genetic variation at various levels.

Phylogenetic analysis

Phylogenetic relationships were analyzed according to the 16S rDNA, *COXI*, and *ITS-2* sequences. Phylogenetic trees were constructed in MEGA-X software with the neighbor-joining algorithm. Branch confidence was validated through 1000 bootstrap replications to ensure the robustness of the tree structure. Additionally, TCS haplotype networks were generated in PopART 1.7 software to visualize relationships among haplotypes, thus aiding in the interpretation of evolutionary connections between populations.

Molecular identification of tick-borne pathogens

To identify pathogens present in tick samples, we performed PCR targeting specific genes for various pathogens: the *gltA* gene for *Rickettsia* [23], the 18S rDNA gene for *Babesia* and *Theileria* [24], the 16S rDNA gene for *A. phagocytophilum* [25], and the *OspA* gene for *B. burgdorferi* [26]. Total tick DNA was used as the template for these assays. The PCR setup was the same as that for tick species identification, and details on reaction conditions and primers are provided in S1 Table. After amplification, pathogen identification was performed via PCR analysis, and the resulting sequences were submitted to the NCBI database for BLASTn analysis to confirm pathogen species. The BLASTn parameters used for pathogen identification were as follows: *Rickettsia*: E-value 0.00–0.01, query coverage 90%–100%, and percentage identity 96%–100% (S5 Table); *Theileria* and *Babesia*: E-value 0.00–0.01, query coverage 96%–100%, and percentage identity 98%–100%.

RESULTS

Tick species identification and distribution

Through a combination of morphological (S1 Fig) and molecular identification techniques, we identified 980 ticks collected from March to July 2021 across 14 prefecture-level cities in the Guangxi Zhuang Autonomous Region, China, as belonging to the Ixodidae family, encompassing two genera: *Rhipicephalus* and *Haemaphysalis*. Among these, *R. microplus* had the highest prevalence (902 individuals representing 92.04% of the sample) and was followed by *Rhipicephalus sanguineus* (*R. sanguineus*) (58, 5.92%), and *Haemaphysalis cornigera* (*H. cornigera*) (20, 2.04%). *R. microplus* demonstrated a widespread distribution across all surveyed cities and was the dominant tick species within the region. In contrast, the distribution of *H. cornigera* was limited to the Liuzhou, Chongzuo, and Yulin populations, whereas *R. sanguineus* was found exclusively in the Laibin population (Fig 1).

Population genetic diversity of *R. microplus*, on the basis of 16S rDNA, *COXI*, and *ITS-2* genes

Because *R. microplus* was identified as the predominant tick species, we performed population diversity analyses based on the 16S rDNA, *COXI*, and *ITS-2* gene sequences. Analysis of 280 mitochondrial 16S rDNA sequences revealed 71 haplotypes (S1–S71) (Fig 2A; S2 Table). Notably, haplotypes S1 and S3 were most prevalent, and accounted for 35.36% and 21.07% of sequences, respectively. The average Hd for 16S rDNA was 0.83, and the Pi was 0.02 (S3 Table). The Hechi population exhibited the highest Hd (0.94), whereas the Chongzuo population had the lowest Hd (0.52). Nucleotide diversity was similarly highest in the Hechi population (Pi = 0.02) and lowest in the Laibin population (Pi = 0.01), and showed significant genetic variability within the *R. microplus* populations.

For the mitochondrial *COXI* gene, 70 haplotypes (C1–C70) were identified (Fig 2B; S2 Table). The haplotype C4, shared across 12 regions in Guangxi, accounted for 43.21% of sequences, thereby suggesting its genetic stability. The overall Hd for *COXI* was 0.81, and the Pi was 0.03 (S3 Table). The Hechi population again showed the highest Hd (1.00), whereas the Chongzuo population had the lowest Hd (0.43). The nucleotide diversity ranged from 0.08 in the Hechi population to 0.0008 in the Laibin population, thereby indicating lower diversity in the *COXI* gene than 16S rDNA.

Analysis of 280 *ITS-2* gene sequences yielded 33 haplotypes (I1–I33), among which I1 was dominant, representing 71.07% of sequences across all 14 regions (Fig 2C; S2 Table). This widespread haplotype suggested low genetic differentiation for the *ITS-2* gene in the region. The overall Hd was 0.49, and the Pi was 0.01. The Hechi population exhibited the highest Hd (0.80), whereas the Guilin population had the lowest Hd (0.10). The Nanning population and Liuzhou population showed the highest (Pi = 0.04) and lowest (Pi = 0.0003) nucleotide diversities, respectively, thus indicating that the *ITS-2* gene had the lowest overall genetic diversity among the three genes analyzed.

Population dynamic history

Neutrality tests for the 16S rDNA and *COXI* genes (Table 1), along with mismatch distribution plots (Fig 3A, B), revealed significant signals of population dynamics. The Guigang population displayed significantly negative Tajima's D and Fu's Fs values, which suggested a recent population expansion. This possibility was further supported by a unimodal peak in the mismatch distribution (Fig 3D, E) indicating population expansion. In contrast, the remaining 13 regions exhibited bimodal patterns indicating genetic stability.

For the *ITS-2* gene, the Guigang, Laibin, Liuzhou, and Qinzhou populations had significantly negative Tajima's D and Fu's Fs values (Table 1), and unimodal peaks in the

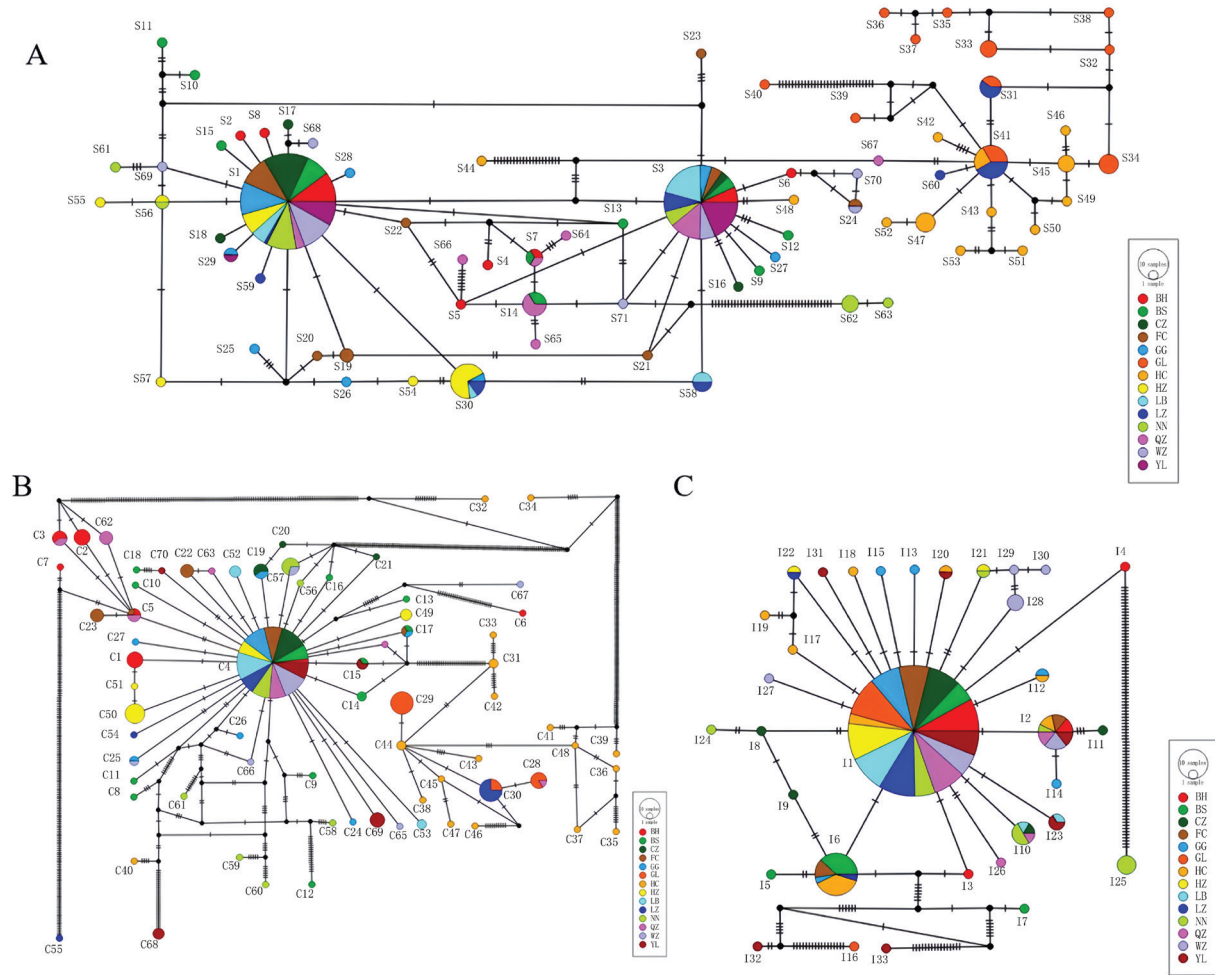


FIGURE 2 | Haplotype network analysis for *R. microplus*. A: Haplotype network constructed on the basis of the mitochondrial 16S rDNA gene. B: Haplotype network constructed on the basis of the *COXI* gene. C: Haplotype network constructed on the basis of the ribosomal *ITS-2* gene.

TABLE 1 | Neutrality test of the 16S rDNA, *COXI*, and *ITS-2* genes.

Population codes ^a	16S rDNA		<i>COXI</i>		<i>ITS-2</i>	
	Tajima's D test	Fu's Fs test	Tajima's D test	Fu's Fs test	Tajima's D test	Fu's Fs test
BH	-0.88510	-2.01813	-1.81103*	2.33169	-1.44071	-2.13527*
BS	-1.07086	-2.47466	-2.21254**	-2.14632	-1.86373*	1.19654
CZ	-1.76227 ^b	-0.57273	-0.97524	-1.40574	-1.90717*	-1.16917
FC	-1.28900	-2.24503	0.33394	0.02831	-0.44022	-0.37748
GG	-1.64420*	-2.87508*	-2.04091**	-3.99996**	-2.05624**	-3.95175**
GL	-1.63885*	-0.71725	1.76767	2.09849	-2.54836**	8.06385
HC	-1.70200*	-3.20953	-1.00917	-1.12922	-1.42693	-3.31888*
HZ	-0.48880	-1.75308	0.15517	-0.26335	-1.72331*	-1.14276
LB	0.96973	0.32450	-0.44022	-0.37748	-1.51284*	-1.86305*
LZ	1.33787	0.76957	-1.45583	23.78613	-1.51284*	-1.86305*
NN	0.55478	9.32446	-1.56234*	2.08053	0.71773	15.72447
QZ	-1.41064	-0.49868	-2.11681**	1.85219	-1.63814*	-1.61348*
WZ	-0.89239	-1.78884	-2.32440**	-0.73956	-0.84629	-1.61327
YL	0.93649	1.54613	-0.29237	8.51117	-1.97677**	2.02306

^aPopulation codes as in Fig 1; ^b $P < 0.05$ *, $P < 0.01$ **.

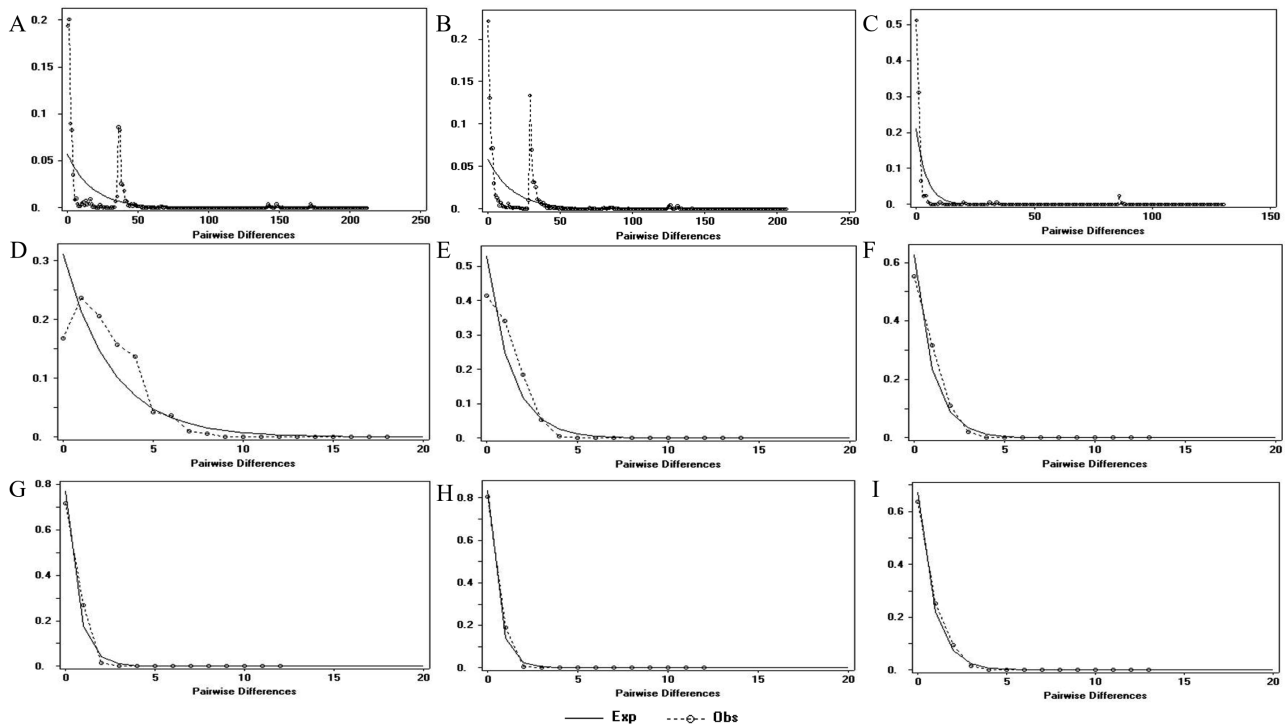


FIGURE 3 | Mismatch distribution map of *R. microplus* populations. A: Mitochondrial 16S rDNA gene. B: Mitochondrial *COXI* gene. C: Ribosomal *ITS-2* gene. D: 16S rDNA gene in the Guigang population (GG). E: *COXI* gene in the Guigang population (GG). F: *ITS-2* gene in the Guigang population (GG). G: *ITS-2* gene in the Laibin population (LB). H: *ITS-2* gene in the Liuzhou population (LZ). I: *ITS-2* gene in the Qinzhou population (QZ). Exp: expected value, Obs: observed value.

mismatch distributions (Fig 3F–I) indicated population expansion in these regions. The other populations showed bimodal peaks suggesting genetic stability.

Population genetic differentiation analysis

Inter-population divergence tests for the 16S rDNA and *COXI* genes (Fig 4A, B) revealed high F_{ST} values (> 0.25) for *R. microplus* populations in Guilin, Hechi, and Liuzhou, thereby indicating significant genetic differentiation. Regions with F_{ST} values below 0.05 showed little to no genetic differentiation, thus indicating frequent gene flow. For the *ITS-2* gene (Fig 4C), all populations had F_{ST} values below 0.25, which suggested no significant genetic differentiation. Populations in Hechi, Nanning, Baise, and Wuzhou exhibited minimal genetic differentiation ($F_{ST} > 0.05$), whereas other regions showed no differentiation; these findings highlighted varying levels of gene flow and genetic structure.

AMOVA based on the 16S rDNA, *COXI*, and *ITS-2* genes (Table 2) indicated both intra- and inter-population variation. For the *COXI* gene, inter-group variance accounted for 52.83% of the total variation and indicated more significant inter-group differences; in contrast, the 16S rDNA and *ITS-2* genes showed higher intra-group variation (63.2% and 87.96%, respectively), thus suggesting that the genetic variation within populations was more significant than that between populations for these genes.

Phylogenetic analysis

Phylogenetic analyses of the 16S rDNA, *COXI*, and *ITS-2* gene sequences (Fig 5) revealed clustering of haplotypes within each gene group, in agreement with the corresponding haplotype networks. However, *R. microplus* did not form a monophyletic group. Notably, genetic analysis found that some haplotypes of *R. microplus* (S62 and S63 from 16S rDNA; C32, C34, and C55 from *COXI*; and I2 from *ITS-2*) were closely related to *Rhipicephalus linnaei*. These findings suggested potential evolutionary relationships between these tick species.

Identification of tick-borne pathogens

In the total collection of 980 ticks, 130 specimens carried one or more pathogens; therefore, the overall infection rate was 13.27%. The infection rates notably varied by location: the Liuzhou population showed the highest rate, at 28.95%, whereas the Laibin population showed the lowest rate, at 9.76%. The detected pathogens included *Rickettsia* (5.10%), *Babesia* (1.12%), and *Theileria* (7.04%) (S4 Table). Co-infection with *Theileria* and *Rickettsia* species was observed in *H. cornigera* from the Liuzhou population and *R. microplus* from the Hechi population, at a co-infection rate of 0.31%.

Genetic sequencing of pathogen-positive samples, compared with sequences in the NCBI database, revealed significant findings. The *gltA* gene sequences of *Rickettsia* showed high homology with *Rickettsia massiliae*

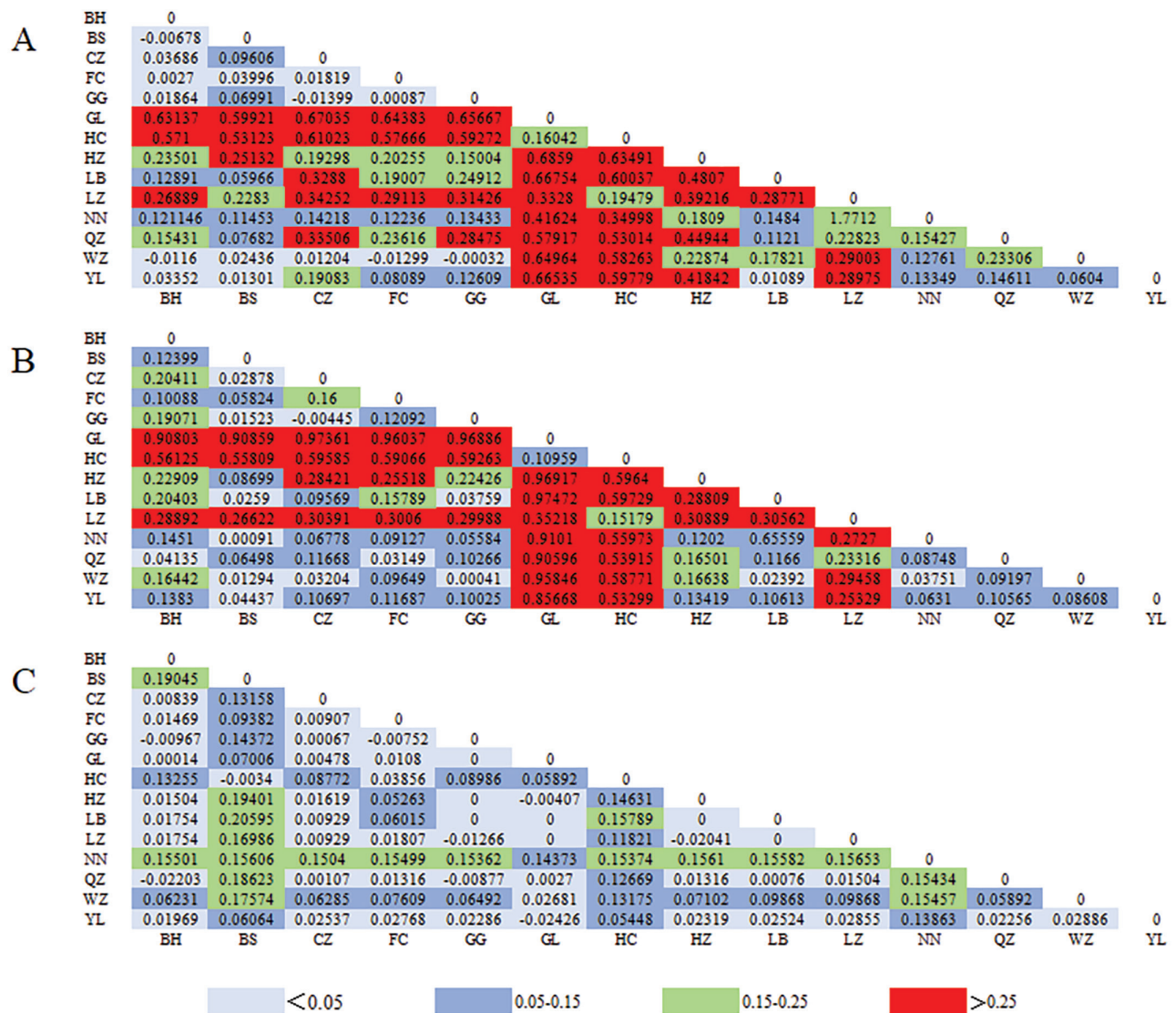


FIGURE 4 | Quantitative assessment of genetic differentiation of *R. microplus* with the fixation index (F_{ST}). A: Mitochondrial 16S rDNA gene. B: Mitochondrial COXI gene. C: Ribosomal ITS-2 gene.

(*R. massiliae*), *Candidatus Rickettsia jingxinensis* (Ca. *R. jingxinensis*), and *Rickettsia conorii* subsp. *Heilongjiangensis* (*R. conorii* subsp. *Heilongjiangensis*), thus indicating the presence of diverse *Rickettsia* species. The 18S rDNA sequences of *Piroplasma* showed genetic similarity to *Theileria orientalis* (*T. orientalis*), *Theileria annulata* (*T. annulata*), *Theileria sinensis* (*T. sinensis*), *Babesia bigemina* (*B. bigemina*), and *Babesia vogeli* (*B. vogeli*).

Phylogenetic analysis of *Rickettsia*, *Babesia*, and *Theileria*

Phylogenetic analyses of *Rickettsia*, *Babesia*, and *Theileria* based on the *gltA* and 18S rDNA genes sequences revealed distinct clustering patterns within each genus. For *Rickettsia*, *gltA* gene analysis showed apparent clustering within the internal groups. Notable genetic variance was observed between *R. massiliae* strains from the Hechi population and Chongzuo population, whereas no

significant divergence was detected between *R. conorii* subsp. *Heilongjiangensis* and *Rickettsia japonica* strains from the Liuzhou population and Chongzuo population (Fig 6A). In contrast, the phylogenetic tree for *Piroplasma* (comprising both *Babesia* and *Theileria*), based on 18S rDNA sequences showed uniform clustering within the internal groups. However, the substantial regional genetic diversity suggested that *Piroplasma* species have followed divergent evolutionary paths across the different geographic regions (Fig 6B).

DISCUSSION

The Guangxi Zhuang Autonomous Region in China, with its warm, humid climate and thriving livestock and tourism industries, has seen increases in tick populations and the risk of tick-borne pathogen transmission, which have posed substantial challenges to the sustainable

TABLE 2 | Molecular variance analysis (AMOVA) based on the 16S rDNA, COXI, and ITS-2 genes.

Gene	Source of variation	Inter-group	Intra-group	Total	F_{ST}
16S rDNA	Degrees of freedom	13	266	279	0.37
	Quadratic sum	299.654	484.85	784.504	
	Variance components	1.06138* Va	1.82274* Vb	2.88412	
	Variation (%)	36.8	63.2	100	
COXI	Degrees of freedom	13	266	279	0.53
	Quadratic sum	1232.454	1077.85	2310.304	
	Variance components	4.53760* Va	4.05207* Vb	8.58967	
	Variation (%)	52.83	47.17	100	
ITS-2	Degrees of freedom	13	266	279	0.12
	Quadratic sum	81.036	443.75	524.786	
	Variance components	0.22826* Va	1.66823* Vb	1.8965	
	Variation (%)	12.04	87.96	100	

Va, inter-group variance component; Vb, intra-group variance component; *significant at $P < 0.01$.

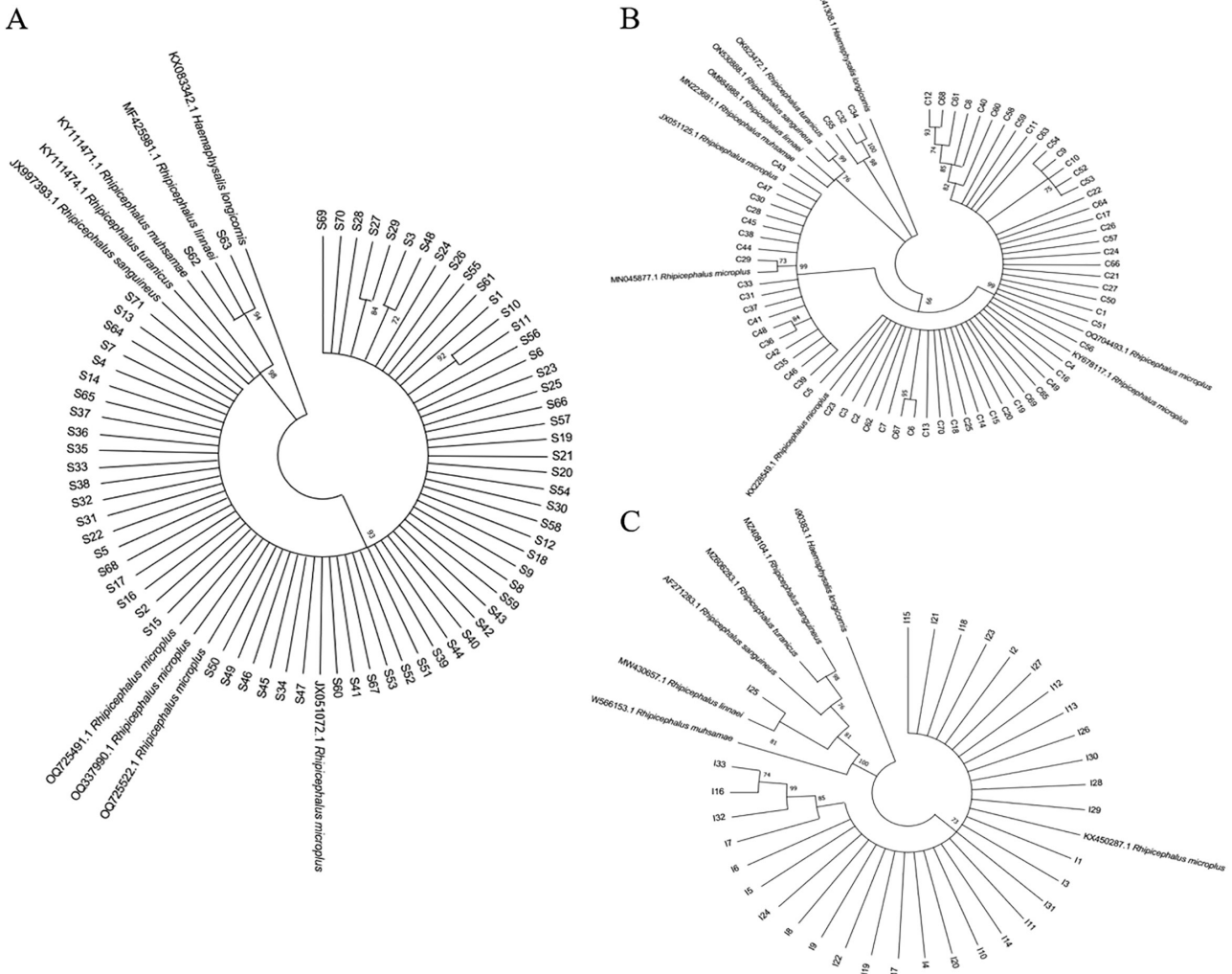


FIGURE 5 | Phylogenetic tree of *R. microplus*. A: Mitochondrial 16S rDNA gene. B: Mitochondrial COXI gene. C: Ribosomal ITS-2 gene.

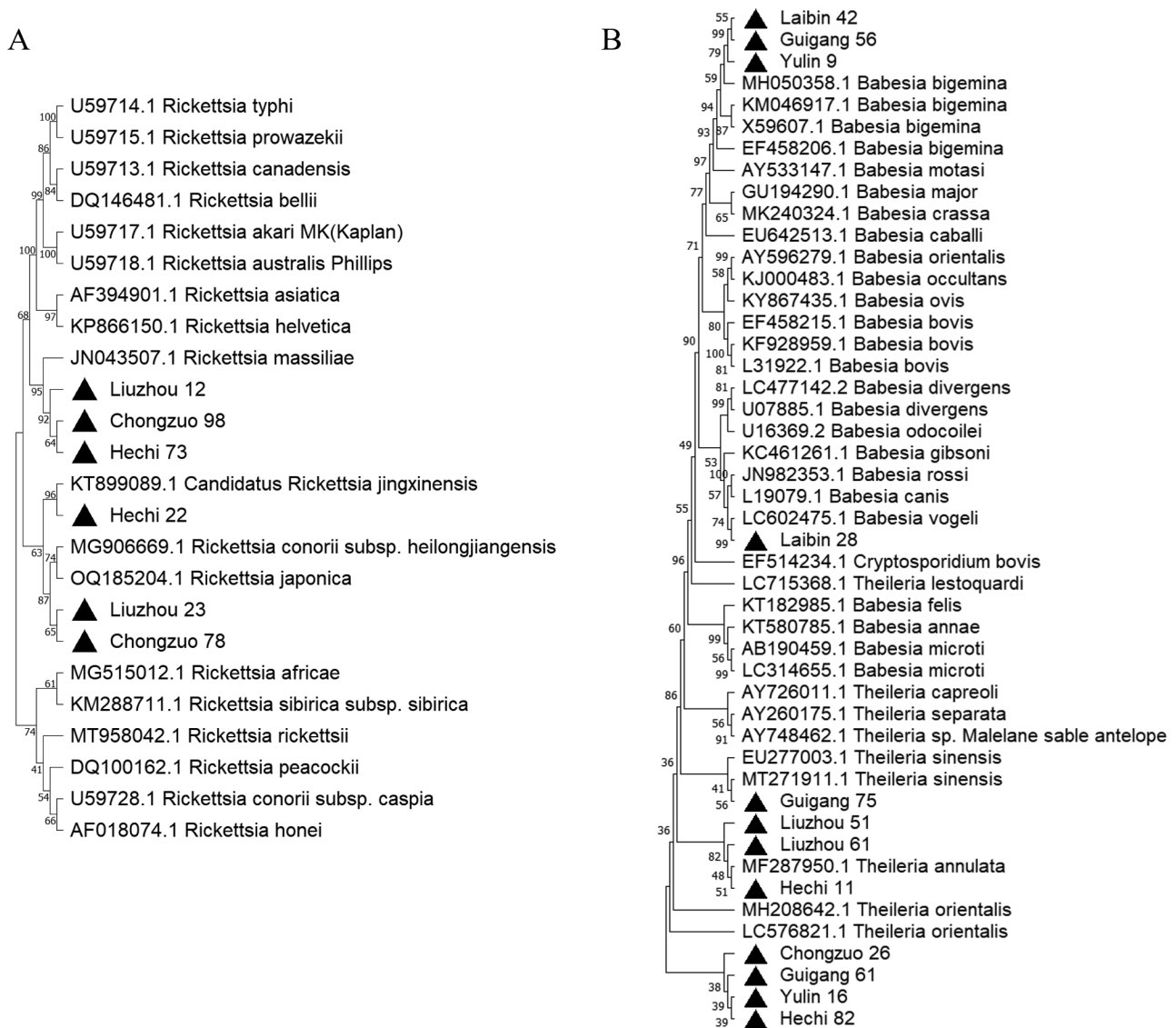


FIGURE 6 | Phylogenetic tree of *Rickettsia*, *Babesia*, and *Theileria*. A: *Rickettsia* phylogenetic tree based on the *gltA* gene. B: Phylogenetic tree of *Babesia* and *Theileria* based on the 18S rDNA gene.

development of these key economic sectors. In this study, we collected 980 tick samples from 14 prefecture-level cities across Guangxi, and identified three species: *R. microplus*, *R. sanguineus*, and *H. cornigera*. The predominant species was *R. microplus*, which accounted for 92.04% of the total samples and was found in all surveyed cities. In addition, we systematically examined the prevalence of five tick-borne pathogens, and identified various species of *Rickettsia*, *Babesia*, and *Theileria*, but did not detect *Borrelia* and *Anaplasma*. Mixed infections with *Theileria* and *Rickettsia* were also observed in specific tick species. To our knowledge, this research is the first systematic examination of the population diversity, genetic structure, and historical dynamics of *R. microplus* in Guangxi, thus providing valuable insights into the ecological and epidemiological factors influencing tick populations in the region.

Population genetic diversity analysis based on the three genes revealed that the Hd of the 16S rDNA and *COXI* genes was higher than that of the *ITS-2* gene. This finding indicated a faster evolutionary rate for 16S rDNA and *COXI* in *R. microplus* than *ITS-2*. Phylogenetic trees constructed from the haplotypes of these genes indicated that the haplotypes for each marker gene consistently clustered together. In 12 *R. microplus* populations, excluding the Guilin and Hechi populations, haplotypes S1, S3, and C4 were shared for the 16S rDNA and *COXI* genes; these findings suggested limited genetic exchange between the Guilin or Hechi populations and those in other regions. For the *ITS-2* gene, at least one shared haplotype (I1) was present in all 14 cities, and the lowest frequency (2.51%) was observed in the Hechi population. The haplotype network analysis suggested that S3, C4, and I1 might be ancient haplotypes in the region. Despite

the complex relationships among haplotypes, the observed links indicated incomplete genetic differentiation among *R. microplus* populations and consequently suggested that the population in Guangxi forms a large, stable group.

Analysis of F_{ST} values derived from the mitochondrial 16S rDNA and *COXI* genes of *R. microplus* populations across 14 regions revealed substantial genetic differentiation and infrequent gene exchange between the populations in Guilin, Hechi and Liuzhou and those in the other regions. This observation aligned with the outcomes of the haplotype analysis. Furthermore, we considered the geographical characteristics of the Guangxi region. The Guilin population and Hechi population were characterized by mountainous and aquatic landscapes. This geographical context suggests potential isolation of the populations in Guilin and Hechi from those in the remaining regions, thus indicating that natural barriers might play major roles in shaping the genetic diversity of *R. microplus* populations within these areas.

Tajima's D and Fu's F_s neutrality tests can be used to analyze the historical dynamics of a population. Only the *R. microplus* population in Guigang exhibited significantly negative values for both Tajima's D and Fu's F_s , alongside a unimodal distribution; therefore, that this population appeared to have experienced expansion. In contrast, populations in other regions appeared to have remained relatively stable throughout their evolutionary history, with no evidence of expansion. However, the *ITS-2* gene results indicated population expansions in Laibin, Liuzhou, and Qinzhou, in addition to Guigang. Because of the *ITS-2* gene's unsuitability for *R. microplus* population analysis, the neutrality test results and mismatch distribution diagrams for the *ITS-2* gene were not considered primary reference data herein.

This study systematically investigated the prevalence of five common tick-borne pathogens. The genus *Rickettsia*, in the Rickettsiaceae family, comprises obligate intracellular Gram-negative bacteria that infect humans, livestock, and wildlife via various arthropod vectors, including ticks, fleas, mites, and lice [27]. Currently, 34 *Rickettsia* species have been officially recognized [28]. In this study, *gltA* gene sequences showed substantial homology with *R. massiliae*, *Ca. R. jingxinensis*, and *R. conorii* subsp. *Heilongjiangensis*, primarily in ticks from the Chongzuo, Hechi, and Liuzhou populations. These findings align with those of Yu et al. and emphasize the urgent need for improved prevention and control measures against rickettsial diseases in these areas [15].

Additionally, this study examined *Piroplasma*, which includes the genera *Babesia* and *Theileria*, both of which pose major health risks to ruminants and humans, particularly in tropical and subtropical regions [29–32]. Sequence analysis revealed that the *Babesia* 18S rDNA sequences, found primarily in *R. microplus* and *R. sanguineus* in Guigang, Yulin, and Laibin, had high homology with *B. bigemina* and *B. vogeli*. Similarly, *Theileria* species, detected primarily in the Chongzuo, Hechi,

Liuzhou, Guigang, and Yulin populations, showed strong homology with *T. orientalis*, *T. annulata*, and *T. sinensis* [33]. The presence of co-infections in ticks suggests elevated risk of complex clinical manifestations in these regions [34–37]. Although mixed infections were relatively rare in Guangxi, shared vectors for multiple pathogens could potentially lead to severe infections in both humans and animals, thereby highlighting the critical need for continuous monitoring and comprehensive strategies to combat TBDs.

This study provides a theoretical reference regarding the species composition, genetic diversity, and potential transmission risk of TBDs in Guangxi, China. However, the study has several limitations. First, because free questing ticks were not collected, the results regarding population genetic diversity and tick-borne pathogens might have been affected. Given that the genetic diversity of different populations and their responses to environmental changes may vary, future studies should expand the source and scope of samples, and enhance the detection of other pathogens, to provide comprehensive and systematic reference data for TBDs prevention and control efforts. Second, this study tested for only five common pathogens but did not detect infection in hosts, and therefore might not fully reflect the potential risk of pathogen transmission by ticks in Guangxi. In future surveillance of tick-borne pathogens, metagenomic detection combined with host antibody testing and other methods should be used to assist pathogen detection, thereby improving the accuracy and comprehensiveness of the results.

CONCLUSION

This study highlights the substantial presence and genetic diversity of *R. microplus* ticks in Guangxi, China, including *Rickettsia*, *Babesia*, and *Theileria*, and emphasizes their roles as vectors for TBDs. The detection of these pathogens, alongside instances of co-infection, underscores the persistent risk of TBDs in the region. The findings indicate the necessity for continuous surveillance, public health education, and integrated tick management strategies to mitigate these risks. The comprehensive epidemiological data provided crucial insights for formulating targeted interventions for the control and prevention of TBDs in Guangxi and beyond, emphasizing a multi-disciplinary approach for effective management.

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CONFLICTS OF INTEREST

We declare no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://zoones-journal.org/wp-content/uploads/2025/01/zoones20240039_Suppl.zip.

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