




Article

Prevalence of Spotted Fever Group *Rickettsia* and *Candidatus Lariskella* in Multiple Tick Species from Guizhou Province, China

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Abstract: Rickettsiales (*Rickettsia* spp., *Ehrlichia* spp., and *Anaplasma* spp., etc.) are generally recognized as potentially emerging tick-borne pathogens. However, some bacteria and areas in China remain uninvestigated. In this study, we collected 113 ticks from mammals in Guizhou Province, Southwest China, and screened for the Rickettsiales bacteria. Subsequently, two spotted fever group *Rickettsia* species and one *Candidatus Lariskella* sp. were detected and characterized. “*Candidatus Rickettsia jingxinensis*” was detected in *Rhipicephalus microplus* (1/1), *Haemaphysalis flava* (1/3, 33.33%), *Haemaphysalis kitaokai* (1/3), and *Ixodes sinensis* (4/101, 3.96%), whereas *Rickettsia monacensis* was positive in *H. flava* (1/3), *H. kitaokai* (2/3), and *I. sinensis* ticks (74/101, 73.27%). At least two variants/sub-genotypes were identified in the *R. monacensis* isolates, and the strikingly high prevalence of *R. monacensis* may suggest a risk of human infection. Unexpectedly, a *Candidatus Lariskella* sp. belonging to the family *Candidatus Midichloriaceae* was detected from *Ixodes ovatus* (1/4) and *I. sinensis* (10/101, 9.90%). The *gltA* and *groEL* gene sequences were successfully obtained, and they show the highest (74.63–74.89% and 73.31%) similarities to “*Candidatus Midichloria mitochondrii*”, respectively. Herein, we name the species “*Candidatus Lariskella guizhouensis*”. These may be the first recovered *gltA* and *groEL* sequences of the genus *Candidatus Lariskella*.

Keywords: *Candidatus Lariskella guizhouensis*; *Rickettsia monacensis*; *Candidatus Rickettsia jingxinensis*; Guizhou Province

1. Introduction

Ticks are one of the most important vectors for pathogens of both humans and animals worldwide [1]. Over the past few decades, more than one hundred tick-borne pathogens, such as spotted fever group *Rickettsia*, tick-borne encephalitis virus (TBEV), and severe fever with thrombocytopenia syndrome virus (SFTSV), have been identified. In recent years, novel tick-borne pathogens have continued to be reported [2,3]. In 2015, a novel *Anaplasma* species named *Anaplasma capra* was detected in 28 patients with symptoms of fever, headache, and malaise in Northeast China [4]. In 2021, a new tick-borne orthonairovirus named Songling virus (SGLV) was identified in patients from Heilongjiang Province, resulting in the infection of 42 hospitalized patients, with headache, fever, fatigue, and dizziness as the main clinical manifestations [5]. In 2021, Kodama et al. reported a novel tick-borne orthonairovirus named Yezo virus (YEZV) in Japan, associated with acute

febrile, thrombocytopenia, and leukopenia [6]. As recently as in 2022, serosurveillance in Japan indicated that a novel tick-borne thogotovirus named Oz virus may naturally infect humans and some other mammals [7]. With increased sampling and development in detection techniques, more novel tick-borne human pathogens are still being discovered.

Due to the vast territory and different climates across China, ticks and tick-borne diseases are prevalent in most areas of this country and pose a significant threat to public health [8]. At least 103 tick-borne agents have been detected, 65 of which were identified in the past two decades [8]. However, most studies have focused on common and easily available tick species, such as *Rhipicephalus microplus*, *Haemaphysalis longicornis*, and *Ixodes persulcatus*. Most of the currently known tick-borne agents were reported in these common tick species [8]. Hence, the pathogens harboured by many tick species may have long been underestimated and largely unexplored due to biased sampling.

The order Rickettsiales includes a group of well-recognized human pathogens such as *Rickettsia* spp., *Orientia* spp., *Ehrlichia* spp., *Anaplasma* spp. and *Neoehrlichia* spp., most of which are tick-borne pathogens [9]. As notorious human pathogens causing a series of symptoms from fever to even death, *Rickettsia*, *Orientia*, *Ehrlichia*, *Neoehrlichia*, and *Anaplasma*, have drawn attention worldwide and been well-studied in many countries and regions [8,10,11]. With the development of high throughput sequencing, some novel families and genera within the order Rickettsiales have been characterized and defined. In 2006, an endosymbiont of the *Ixodes ricinus* named “*Candidatus* Midichloria mitochondrii” was described, which also represents a novel genus *Candidatus* Midichloria within the order Rickettsiales [12]. In 2013, a novel family named “*Candidatus* Midichloriaceae” was proposed, including genera *Candidatus* Midichloria, *Candidatus* Lariskella, *Candidatus* Nicolleia, *Candidatus* Fokinia, and *Candidatus* Aquarickettsia [13]. Although some have been reported to infect humans and mammals such as dogs and horses [14,15], studies on the prevalence, genetic diversity, and pathogenicity of these bacteria are very rare.

To improve the current knowledge on the distribution of ticks and tick-borne Rickettsiales pathogens, ticks collected from Qiandongnan Miao-and-Dong Autonomous Prefecture of Guizhou Province, Southwest China, were thoroughly investigated.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

From November to October 2021, ticks were collected in Cengong County (108.82 °E, 27.18 °N), Qiandongnan Miao-and-Dong Autonomous Prefecture, located in Guizhou Province, Southwest China. All the ticks were removed from the body surface of goats and dogs using tweezers, and then stored in 75% alcohol. Morphological identification, mainly based on the characteristic of the anal groove, was carried out by an experienced acarologist to determine the tick species initially. To confirm these results, molecular identification was performed by PCR amplifying and sequencing the partial mitochondrial cytochrome oxidase I (COI) gene of randomly selected tick samples, as described previously [14]. After being washed with Phosphate Buffered Saline (PBS) and thoroughly ground, each tick was subjected to DNA extraction individually using a Mollusc DNA extraction kit (Omega, Norcross, GA, USA) following the instructions. The eluted DNA (60 µL) was stored in a −80 °C refrigerator until molecular identification and PCR detection.

2.2. Molecular Detection of Rickettsiales Bacteria

The extracted DNA was screened by PCR analysis of a conserved region of *rrs* gene to confirm that the bacteria belong to the order Rickettsiales. PCR was performed using Sensoquest PCR System LabCycler (Sensoquest, Göttingen, Germany). As previously shown, PCR amplification screening *Rickettsia* generates approximately 900 bp products, whereas PCR detecting Anaplasmataceae bacteria generates approximately 450 bp products [16]. The DNA of *R. japonica* and *A. marginale* were used as positive controls, whereas ddH₂O was set as the negative control. After electrophoresis, all the PCR products that met the

expected size were subjected to DNA sequencing. The recovered *rrs* sequences were then aligned to those in the GenBank Database to preliminarily determine their genus or species.

2.3. PCR Amplification, Sequencing, Genetic and Phylogenetic Analysis of Key Genes

The citrate synthase gene (*gltA*, 996 bp), 60 kDa chaperonin gene (*groEL*, 1026–1030 bp), outer membrane protein A gene (*ompA*, 706–718 bp), as well as a longer fragment of *rrs* gene (1214–1215 bp), were successfully amplified from the randomly selected *Rickettsia*-positive samples using primers as shown previously [16,17]. For the detected *Candidatus* Lariskella isolates, primers amplifying the *gltA* (456 bp) and *groEL* (651 bp) genes were designed (Table S1). The primers were based on the nucleotide sequences of other *Candidatus* Midichloriaceae members (“*Candidatus* Midichloria mitochondrii”, “*Candidatus* Fokinia cryptica”, and “*Candidatus* Jidaibacter acanthamoeba”), due to the absence of sequences from the genus *Candidatus* Lariskella.

The obtained sequences were aligned with the reference sequences in the GenBank Database with BLASTn algorithm to determine their identities to the reported strains. To perform the phylogenetic analysis, the nucleotide sequences of *Rickettsia* isolates were first manually aligned with those downloaded from the GenBank using the ClustalW method in the MEGA software v3.0. For the *Candidatus* Lariskella isolates, the *rrs* sequences were aligned with other *Candidatus* Lariskella isolates and some *Candidatus* Midichloriaceae members, whereas the *gltA* and *groEL* sequences were only aligned with other *Candidatus* Midichloriaceae members and some Rickettsiaceae members due to the unavailability of *Candidatus* Lariskella sequences in GenBank. Phylogenetic trees were constructed based on the maximum likelihood (ML) method by PhyML v3.2 in the GTR+I+G model [18]. The confidence values for each branch were determined by bootstrap analysis with 100 repetitions. All the trees were mid-point rooted and only bootstrap values >70% are shown.

All the sequences in this study have been uploaded to the GenBank Database (shown in Table S2).

3. Results

3.1. Sample Collection and Tick Identification

From November to October 2021, a total of 113 ticks were collected (112 from goats and 1 from a dog) in Cengong County, Qiandongnan Prefecture. Morphological and molecular identification confirmed the existence of 6 tick species: 1 *Rhipicephalus microplus*, 1 *Haemaphysalis longicornis*, 3 *Haemaphysalis flava*, 3 *Haemaphysalis kitaokai*, 4 *Ixodes ovatus*, and 101 *Ixodes sinensis*. BLASTn shows that all the obtained COI sequences (GenBank Accession Numbers: OP107272–OP107273, OP107278–OP107354) have higher than 99% identities to reference COI sequences in the GenBank except for the *I. ovatus* ticks which have lower identities of approximately 96%. This is consistent with previous reports that *I. ovatus* contain different phylogenetic groups with remarkable intergroup genetic distances [19]. To our knowledge, this is the first report that *H. flava*, *H. kitaokai*, *I. ovatus*, and *I. sinensis* are present in Guizhou Province. Phylogenetic analysis results are consistent with the BLASTn results (Figure 1), indicating the remarkable diversity of tick species in this area.

3.2. Detection and Characterization of *Rickettsia* Isolates

Based on DNA sequencing and sequence analysis, a total of two *Rickettsia* species were initially determined: *Rickettsia monacensis* and “*Candidatus* *Rickettsia* jingxinensis”. “*Candidatus* *Rickettsia* jingxinensis” was detected in seven tick samples: *R. microplus* (1/1, 100%), *H. flava* (1/3, 33.33%), *H. kitaokai* (1/3, 33.33%), and *I. sinensis* (4/101, 3.96%) (Table 1). The *rrs* (1214 bp), *gltA* (996 bp), *groEL* (1026 bp), and *ompA* (706 bp) gene sequences of the two isolates were all identical to the reported “*Candidatus* *Rickettsia* jingxinensis” isolates from elsewhere in China.

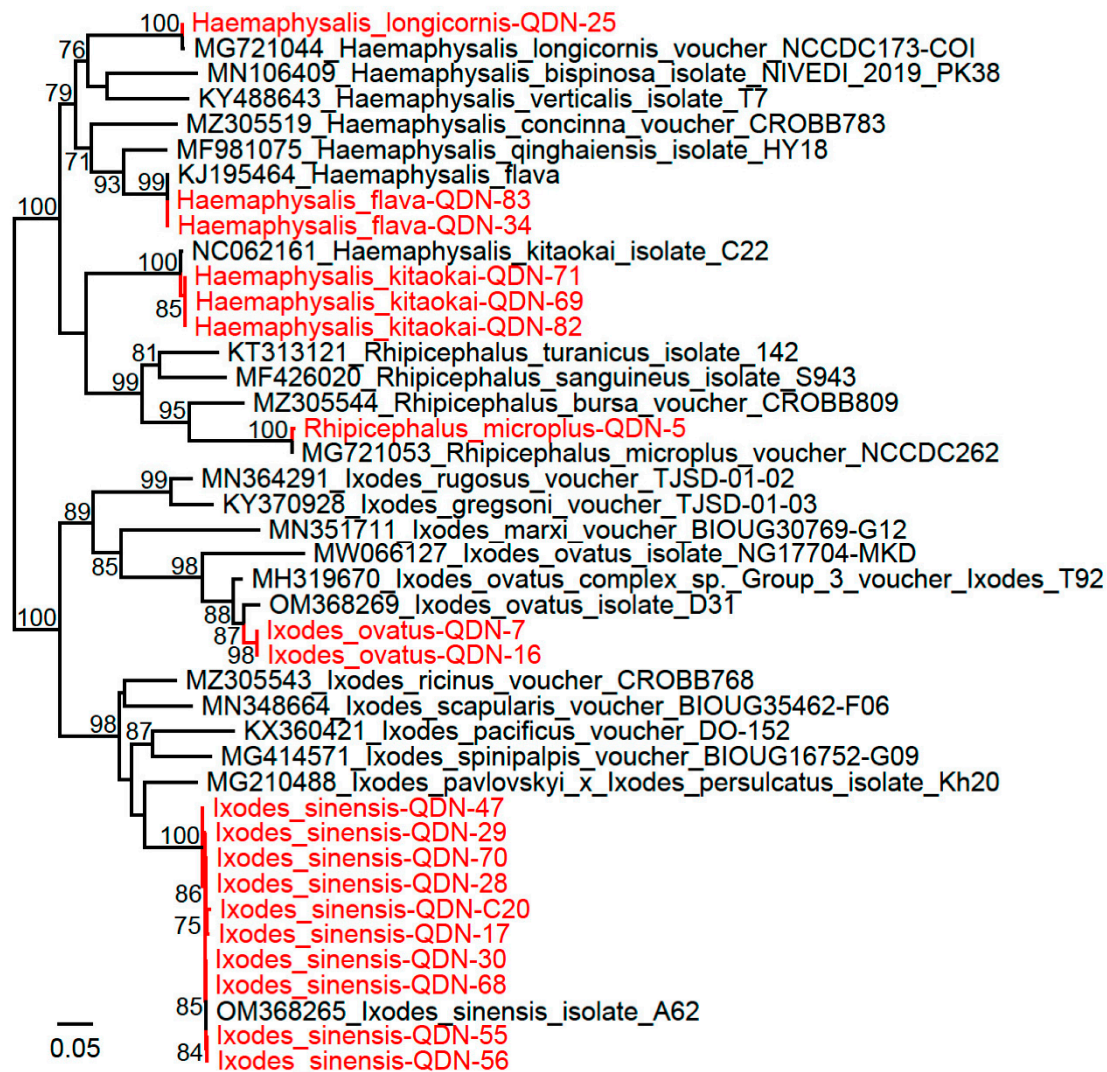


Figure 1. Phylogenetic trees based on the COI sequences of ticks from Guizhou Province. Red: Sequences obtained in this study.

Table 1. Prevalence of *Rickettsia* and “*Candidatus* *Lariskella guizhouensis*” in different tick species from Guizhou Province.

	“ <i>Ca. Rickettsia</i> <i>Jingxinensis</i> ”	<i>Rickettsia</i> <i>monacensis</i>	“ <i>Ca. Lariskella</i> <i>Guizhouensis</i> ”
<i>R. microplus</i>	1/1 (100%) ^a	0/1 (0.00%)	0/1 (0.00%)
<i>H. longicornis</i>	0/1 (0.00%)	0/1 (0.00%)	0/1 (0.00%)
<i>H. flava</i>	1/3 (33.33%)	1/3 (33.33%)	0/1 (0.00%)
<i>H. kitaokai</i>	1/3 (33.33%)	2/3 (66.67%)	0/3 (0.00%)
<i>I. ovatus</i>	0/4 (0.00%)	0/4 (0.00%)	1/4 (25.00%)
<i>I. sinensis</i>	4/101 (3.96%)	74/101 (73.27%)	10/101 (9.90%)
Total	7/113 (6.19%)	77/113 (68.14%)	11/113 (9.73%)

^a positive samples/total samples.

A total of 77 ticks were positive for *R. monacensis* including *H. flava* (1/3, 33.33%), *H. kitaokai* (2/3, 66.67%), and *I. sinensis* (74/101, 73.27%) (Table 1). The *rrs* gene (1215 bp) sequences were identical to strains (KX987304–KX987306) previously identified in *I. sinensis* from Wuhan City, Hubei Province, China. The *groEL* sequences (1030 bp) have 99.61–99.71% identity to *R. monacensis* strain IrR/Munich (LN794217). Interestingly, for the *gltA* (996 bp)

16S

94 CP000087_Rickettsia_bellii_RML369-C
LN829697_Rickettsia_endosymbiont_of_Bemisia_tabaci
AF503168_Candidatus_Rickettsia_tarasevichiae
CP003304_Rickettsia_canadensis_str_CA410
NR074394_Rickettsia_typhi_Wilmington
NR041840_Rickettsia_asiatica_strain_IO-1
CP000053_Rickettsia_felis_URRWXCal2
CP003338_Rickettsia_australis_str_Cutlack
U12458_Rickettsia_akari_Hartford
100 NR115686_Rickettsia_monacensis_strain_Ir/Munich
Rickettsia_monacensis_QDN-C14
Rickettsia_monacensis_QDN-C7
Rickettsia_monacensis_QDN-C4
Rickettsia_monacensis_QDN-1
Rickettsia_monacensis_QDN-3
NR025920_Rickettsia_montanensis_strain_ATCC_VR-611
MK304546_Rickettsia_raoultii_isolate_Tomsk
NR041770_Rickettsia_heliogiangensis_strain_HLJ-054
NC016050_Rickettsia_japonica_YH
94 MH500199_Candidatus_Rickettsia_jingxinensis_isolate_tick-XA188
Candidatus_Rickettsia_jingxinensis_QDN-5
Candidatus_Rickettsia_jingxinensis_QDN-83
NR026042_Rickettsia_aeschlmannii_strain_MC16
NR025921_Rickettsia_rhipicephali_strain_3-7-6
CP000683_Rickettsia_massilliae_MTU5
U12462_Rickettsia_sibirica_246
CP000766_Rickettsia_rickettsii_str_lowa
NR029156_Rickettsia_parkeri_strain_Maculatum
NC003103_Rickettsia_conorii_strain_Malish_7
NC016639_Rickettsia_slovaca_strain_13-B

0.002

groEL

100 CP000087_Rickettsia_bellii_RML369-C
EU435143_Rickettsia_endosymbiont_of_Bemisia_tabaci_str_CA410
CP003304_Rickettsia_canadensis_str_CA410
97 AP019563_Rickettsia_asiatica_Maytar01284
DQ442911_Rickettsia_helvetic
100 LN794217_Rickettsia_monacensis_strain_Ir/Munich
Rickettsia_monacensis_QDN-3
Rickettsia_monacensis_QDN-1
Rickettsia_monacensis_QDN-C7
Rickettsia_monacensis_QDN-C4
Rickettsia_monacensis_QDN-C14
88 AE017197_Rickettsia_typhi_str_Wilmington
CP004889_Rickettsia_prowazekii_str_Breiln
95 JSELO1000012_Rickettsia_felis
74 CP000847_Rickettsia_akari_str_Hartford
CP003338_Rickettsia_australis_str_Cutlack
91 CP019435_Rickettsia_raoultii_strain_IM16
CP003340_Rickettsia_montanensis_str_OSU_85-930
89 CP000683_Rickettsia_massilliae_MTU5
CP003342_Rickettsia_rhipicephali_str_3-7-female6-CWPP
Candidatus_Rickettsia_jingxinensis_QDN-5
Candidatus_Rickettsia_jingxinensis_QDN-83
76 AP019865_Rickettsia_heliogiangensis_Sendai-58
AP011533_Rickettsia_japonica_strain_YH
94 CP000766_Rickettsia_rickettsii_str_lowa
NC012730_Rickettsia_peacockii_str_Rustic
88 CP002428_Rickettsia_slovaca_13-B
77 AE006914_Rickettsia_conorii_str_Malish_7
90 NC017044_Rickettsia_parkeri_str_Portsmouth
CP001612_Rickettsia_africae_ESF-5

0.01

gltA

CP000087_Rickettsia_bellii_RML369-C
CP003304_Rickettsia_canadensis_str_CA410
MK923733_Rickettsia_assembionis_strain_LER197
JQ674484_Rickettsia_felis
98 CP003338_Rickettsia_australis_str_Cutlack
CP000847_Rickettsia_akari_str_Hartford
100 U59715_Rickettsia_prowazekii_str_Breiln
U59714_Rickettsia_typhi_str_Wilmington
80 Rickettsia_monacensis_QDN-C14
CBUA01000018_Rickettsia_monacensis_Ir/Munich
Rickettsia_monacensis_QDN-3
Rickettsia_monacensis_QDN-C7
91 Rickettsia_monacensis_QDN-1
Rickettsia_monacensis_QDN-C4
78 AP019563_Rickettsia_asiatica_Maytar01284
CP003340_Rickettsia_montanensis_str_OSU_85-930
CP010969_Rickettsia_raoultii_strain_Khabarovsk
95 CP000683_Rickettsia_massilliae_MTU5
CP003342_Rickettsia_rhipicephali_str_3-7-female6-CWPP
73 CP001227_Rickettsia_peacockii_str_Rustic
CP003308_Rickettsia_philipi_str_364D
96 CP000766_Rickettsia_rickettsii_str_lowa
CP003341_Rickettsia_parkeri_str_Portsmouth
AF018074_Rickettsia_honei
AE006914_Rickettsia_conorii_str_Malish_7
CP002428_Rickettsia_slovaca_13-B
CP001612_Rickettsia_africae_ESF-5
85 Candidatus_Rickettsia_jingxinensis_QDN-5
MH932024_Candidatus_Rickettsia_jingxinensis_isolate_Xian-HI-79
94 AP011533_Rickettsia_japonica_YH
76 AP019862_Rickettsia_heliogiangensis_CH8-1

0.02

ompA

CP012420_Rickettsia_amblyommatis_strain_Ac37
CP010969_Rickettsia_raoultii_strain_Khabarovsk
99 U43803_Rickettsia_rhipicephali_str_3-7-female6-CWPP
CP000683_Rickettsia_massilliae_MTU5
CP003340_Rickettsia_montanensis_str_OSU_85-930
91 DQ103259_Rickettsia_tamurae_strain_AT-1
LN794217_Rickettsia_monacensis_strain_Ir/Munich
100 Rickettsia_monacensis_QDN-C14
Rickettsia_monacensis_QDN-3
Rickettsia_monacensis_QDN-1
Rickettsia_monacensis_QDN-C7
79 EU665233_Rickettsia_monacensis
Rickettsia_monacensis_QDN-C4
GQ223392_Candidatus_Rickettsia_tasmanensis_strain_T152
92 AP011533_Rickettsia_japonica_YH
99 MH932069_Candidatus_Rickettsia_jingxinensis_isolate_Xian-HI-79
Candidatus_Rickettsia_jingxinensis_QDN-83
Candidatus_Rickettsia_jingxinensis_QDN-5
96 CP001227_Rickettsia_peacockii_str_Rustic
EU272186_Candidatus_Rickettsia_barbariae
CP001612_Rickettsia_africae_ESF-5
87 CP003341_Rickettsia_parkeri_str_Portsmouth
91 AE006914_Rickettsia_conorii_str_Malish_7
CP002428_Rickettsia_slovaca_13-B
75 CP000766_Rickettsia_rickettsii_str_lowa
99 CP003308_Rickettsia_philipi_str_364D

0.02

3.3. Detection and Characterization of *Candidatus Lariskella* Isolates

Electrophoresis indicated that 11 tick samples were positive for the Anaplasmataceae bacteria. Unexpectedly, sequencing results showed that all of them belong to the genus *Candidatus* Lariskella, the family *Candidatus* Midichloriaceae. Of the 11 isolates, 1 was from *I. ovatus* (1/4, 25.00%) and 10 were from *I. sinensis* (10/101, 9.90%) (Table 1). The amplified *rrs* sequences (468 bp) have the highest (99.36–99.57%) identity to *Candidatus* Lariskella sp. isolates identified in *I. sinensis* from Wuhan City (KX987316, KX987317) and 98.72–98.93%

to the “*Candidatus Lariskella arthropodarum*” clone AmLaKka1 (JQ726736) detected in *Arocatas melanostomus* from Japan.

For further study of the detected *Candidatus Lariskella* isolates, we tentatively designed primers to amplify the *gltA* and *groEL* genes based on sequences of other genera of the family *Candidatus Midichloriaceae*. Subsequently, both the *gltA* (410–453 bp) and *groEL* (651 bp) were successfully recovered from four randomly selected isolates. BLASTn alignment showed that the two gene sequences were mostly related to “*Candidatus Midichloria mitochondrii*”. The *gltA* sequences have 74.63–74.89% identity to “*Candidatus Midichloria mitochondrii*” IricVA (CP002130), and 74.63–74.71% identity to “*Candidatus Nicolleia masiliensis*” (DQ788563), both of which belong to the family *Candidatus Midichloriaceae*, the order Rickettsiales. Meanwhile, the *groEL* sequences showed 73.31% identity to “*Candidatus Midichloria mitochondrii*” IricVA (CP002130), and 71.21% to Rickettsiales bacterium Ac37b (CP009217). To the best of our knowledge, these may be the first obtained *gltA* and *groEL* sequences of the genus *Candidatus Lariskella*. In the phylogenetic tree based on the *gltA* and *groEL* genes, all four isolates were surrounded by other *Candidatus Midichloriaceae* members as well as several Rickettsiaceae members, but they were distinct from any other bacterial species and formed an independent clade (Figure 3). All of these results indicated the representation of an unrecognized species. Herein, we name it “*Candidatus Lariskella guizhouensis*”.



Figure 3. Phylogenetic trees based on the nucleotide sequences of 16S rRNA (*rrs*), *gltA*, and *groEL* genes of the *Candidatus Lariskella* isolates. Red: Sequences obtained in this study.

4. Discussions

To date, approximately 124 tick species belonging to 11 genera have been recorded in China [8]. Although the geographic distribution of various tick species has been well studied, some neglected areas are still largely unexplored. Herein, we collected 113 ticks in Guizhou Province, Southwest China, and molecularly validated the existence of six tick species in them. Of those, *I. sinensis*, *H. flava*, *H. kitaokai*, and *I. ovatus* had never been recorded in this province. These results may contribute to our knowledge of the geographic distribution of ticks and tick-borne pathogens in China.

Two *Rickettsia* species were detected in tick samples: *Rickettsia monacensis* and “*Candidatus Rickettsia jingxinensis*”. As a widely distributed spotted fever group *Rickettsia*, “*Candidatus Rickettsia jingxinensis*” has been detected in *R. microplus* and *H. longicornis* from multiple provinces in China (Liaoning, Guangxi, Sichuan, Hebei, Shaanxi, and Yunnan) and other Asian countries (Korea and India) [16,17,20–22]. Our results have demonstrated the circulation of “*Candidatus Rickettsia jingxinensis*” in Guizhou Province. Due to its wide geographic distribution and host range, the potential risk to the public health of this *Rickettsia* should be evaluated. In this study, *R. monacensis* was detected in three tick species with a total prevalence of 68.14%. Due to the fact that all the ticks were removed from domestic animals, the possibility remains that the detected pathogens are from the blood of infected hosts the ticks fed on. *Rickettsia monacensis* was first identified in *Ixodes ricinus* ticks from Germany [23] and has also been detected in other tick species such as *Ixodes boliviensis*, *I. sinensis*, *Rhipicephalus sanguineus*, *Hyalomma impeltatum* [24–26], as well as some mammal

hosts such as camels and bats [26,27]. As an SFGR member, *R. monacensis* was recognized as a human pathogen causing Mediterranean Spotted Fever-like rickettsioses [25]. Although *R. monacensis* has been detected in several provinces of China [25,28,29], no human infection cases were reported until now. The strikingly high prevalence of *R. monacensis* in ticks (especially in *I. sinensis*) may indicate the risk of human infection, especially in populations who are frequently in contact with domestic animals. Furthermore, genetic and phylogenetic analysis indicated that two variants/sub-genotypes of the *R. monacensis* isolates may have been generated, indicating its long-term evolution in this area. The pathogenicity of the variants/sub-genotypes may warrant further studies.

First proposed in 2012, the genus *Candidatus Lariskella* has been scarcely studied [30], and little is known about its genetic features. Even with respect to the first identified and most studied species, “*Candidatus Lariskella arthropodarum*” (previously named Montezuma) [14], only some *rrs* sequences were available in the GenBank Database until now. Notably, “*Candidatus Lariskella arthropodarum*” has been detected in acutely febrile patients with the bites of *Ixodes* ticks in the Far East of Russia [14], suggesting its potential role as a tick-borne human pathogen. In this study, we detected a *Candidatus Lariskella* species in *Ixodes* ticks from Guizhou Province, and successfully obtained the *gltA* and *groEL* sequences. To our knowledge, these are the first reported *gltA* and *groEL* sequences of the genus *Candidatus Lariskella*. The remarkable genetic distance between this species and other *Candidatus Lariskella* species indicates that it represents a novel species. Herein we named it “*Candidatus Lariskella guizhouensis*”. It is of interest whether “*Candidatus Lariskella guizhouensis*” can be transmitted to humans and animals through tick bites.

5. Conclusions

In conclusion, a high prevalence of *Rickettsia monacensis* was observed in ticks from Guizhou Province, and they represent at least two variants/sub-genotypes. For this reason, the risk of human infection among local people should be evaluated. In addition, a *Candidatus Lariskella* sp. belonging to the family *Candidatus Midichloriaceae* was detected and characterized. To the best of our knowledge, this is the first report of *gltA* and *groEL* sequences of *Candidatus Lariskella*. Genetic and phylogenetic analysis indicates that it represents a novel species: “*Candidatus Lariskella guizhouensis*”. This study may contribute to our knowledge of the extensive genetic diversity of Rickettsiales bacteria, and highlights that their potential threat to human health should be further investigated.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biom12111701/s1>, Table S1. The primers used for amplification of *gltA* and *groEL* genes from *Candidatus Lariskella guizhouensis* by hemi-nested PCR. Table S2. Genbank numbers of the *rrs*, *gltA*, *groEL*, and *ompA* sequences of *Rickettsia* and “*Candidatus Lariskella guizhouensis*” in this study.

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References

- Mansfield, K.L.; Jizhou, L.; Phipps, L.P.; Johnson, N. Emerging tick-borne viruses in the twenty-first century. *Front. Cell Infect. Microbiol.* **2017**, *7*, 298. [\[CrossRef\]](#) [\[PubMed\]](#)
- Liu, Q.; He, B.; Huang, S.Y.; Wei, F.; Zhu, X.Q. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. *Lancet Infect. Dis.* **2014**, *14*, 763–772. [\[CrossRef\]](#)
- Dehghani, M.; Kazemi Shariat Panahi, H.; Holmes, E.C.; Hudson, B.J.; Schloeffel, R.; Guillemin, G.J. Human tick-borne diseases in Australia. *Front. Cell Infect. Microbiol.* **2019**, *9*, 3. [\[CrossRef\]](#)
- Li, H.; Zheng, Y.C.; Ma, L.; Jia, N.; Jiang, B.G.; Jiang, R.R.; Huo, Q.B.; Wang, Y.W.; Liu, H.B.; Chu, Y.L.; et al. Human infection with a novel tick-borne Anaplasma species in China: A surveillance study. *Lancet Infect. Dis.* **2015**, *15*, 663–670. [\[CrossRef\]](#)
- Ma, J.; Lv, X.L.; Zhang, X.; Han, S.Z.; Wang, Z.D.; Li, L.; Sun, H.T.; Ma, L.X.; Cheng, Z.L.; Shao, J.W.; et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat. Med.* **2021**, *27*, 434–439. [\[CrossRef\]](#)
- Kodama, F.; Yamaguchi, H.; Park, E.; Tatemoto, K.; Sashika, M.; Nakao, R.; Terauchi, Y.; Mizuma, K.; Orba, Y.; Kariwa, H.; et al. A novel nairovirus associated with acute febrile illness in Hokkaido, Japan. *Nat. Commun.* **2021**, *12*, 5539. [\[CrossRef\]](#) [\[PubMed\]](#)
- Tran, N.T.B.; Shimoda, H.; Ishijima, K.; Yonemitsu, K.; Minami, S.; Kuroda, Y.; Tatemoto, K.; Mendoza, M.V.; Kuwata, R.; Takano, A.; et al. Zoonotic infection with Oz virus, a novel Thogotovirus. *Emerg. Infect. Dis.* **2022**, *28*, 436–439. [\[CrossRef\]](#)
- Zhao, G.P.; Wang, Y.X.; Fan, Z.W.; Ji, Y.; Liu, M.J.; Zhang, W.H.; Li, X.L.; Zhou, S.X.; Li, H.; Liang, S.; et al. Mapping ticks and tick-borne pathogens in China. *Nat. Commun.* **2021**, *12*, 1075. [\[CrossRef\]](#) [\[PubMed\]](#)
- Matos, A.L.; Curto, P.; Simões, I. Moonlighting in Rickettsiales: Expanding virulence landscape. *Trop. Med. Infect. Dis.* **2022**, *7*, 32. [\[CrossRef\]](#) [\[PubMed\]](#)
- Low, V.L.; Tan, T.K.; Khoo, J.J.; Lim, F.S.; AbuBakar, S. An overview of rickettsiae in Southeast Asia: Vector-animal-human interface. *Acta Trop.* **2020**, *202*, 105282. [\[CrossRef\]](#) [\[PubMed\]](#)
- Silaghi, C.; Beck, R.; Oteo, J.A.; Pfeffer, M.; Sprong, H. Neorickettsiosis: An emerging tick-borne zoonosis caused by *Candidatus Neorickettsia mikurensis*. *Exp. Appl. Acarol.* **2016**, *68*, 279–297. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sassera, D.; Beninati, T.; Bandi, C.; Bouman, E.A.P.; Sacchi, L.; Fabbri, M.; Lo, N. ‘*Candidatus Midichloria mitochondrii*’, an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2535–2540. [\[CrossRef\]](#) [\[PubMed\]](#)
- Montagna, M.; Sassera, D.; Epis, S.; Bazzocchi, C.; Vannini, C.; Lo, N.; Sacchi, L.; Fukatsu, T.; Petroni, G.; Bandi, C. “*Candidatus Midichloriaceae*” fam. nov. (Rickettsiales), an ecologically widespread clade of intracellular alphaproteobacteria. *Appl. Environ. Microbiol.* **2013**, *79*, 3241–3248. [\[CrossRef\]](#) [\[PubMed\]](#)
- Mediannikov, O.I.; Ivanov, L.I.; Nishikawa, M.; Saito, R.; Sidel’nikov, I.N.; Zdanovskaia, N.I.; Mokretsova, E.V.; Tarasevich, I.V.; Suzuki, H. Microorganism “Montezuma” of the order Rickettsiales: The potential causative agent of tick-borne disease in the Far East of Russia. *Z. Mikrobiol. Epidemiol. Immunobiol.* **2004**, *1*, 7–13.
- Bazzocchi, C.; Mariconti, M.; Sassera, D.; Rinaldi, L.; Martin, E.; Cringoli, G.; Urbanelli, S.; Genchi, C.; Bandi, C.; Epis, S. Molecular and serological evidence for the circulation of the tick symbiont *Midichloria* (Rickettsiales: Midichloriaceae) in different mammalian species. *Parasit. Vectors.* **2013**, *6*, 350. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lu, M.; Tian, J.; Pan, X.; Qin, X.; Wang, W.; Chen, J.; Guo, W.; Li, K. Identification of *Rickettsia* spp., *Anaplasma* spp., and an *Ehrlichia canis*-like agent in *Rhipicephalus microplus* from Southwest and South-Central China. *Ticks Tick Borne Dis.* **2022**, *13*, 101884. [\[CrossRef\]](#)
- Lu, M.; Tian, J.; Zhao, H.; Jiang, H.; Qin, X.; Wang, W.; Li, K. Molecular survey of vector-borne pathogens in ticks, sheep keds, and domestic animals from Ngawa, Southwest China. *Pathogens* **2022**, *11*, 606. [\[CrossRef\]](#) [\[PubMed\]](#)
- Guindon, S.; Delsuc, F.; Dufayard, J.F.; Gascuel, O. Estimating maximum likelihood phylogenies with PhyML. *Methods Mol. Biol.* **2009**, *537*, 113–137. [\[CrossRef\]](#)
- Li, L.H.; Zhang, Y.; Wang, J.Z.; Li, X.S.; Yin, S.Q.; Zhu, D.; Xue, J.B.; Li, S.G. High genetic diversity in hard ticks from a China-Myanmar border county. *Parasit. Vectors.* **2018**, *11*, 469. [\[CrossRef\]](#)
- Bang, M.S.; Kim, C.M.; Pyun, S.H.; Kim, D.M.; Yun, N.R. Molecular investigation of tick-borne pathogens in ticks removed from tick-bitten humans in the southwestern region of the Republic of Korea. *PLoS ONE* **2021**, *16*, e0252992. [\[CrossRef\]](#)
- Liu, H.; Liang, X.; Wang, H.; Sun, X.; Bai, X.; Hu, B.; Shi, N.; Wang, N.; Zhang, X.; Huang, L.; et al. Molecular evidence of the spotted fever group Rickettsiae in ticks from Yunnan Province, Southwest China. *Exp. Appl. Acarol.* **2020**, *80*, 339–348. [\[CrossRef\]](#)
- Guo, W.P.; Wang, Y.H.; Lu, Q.; Xu, G.; Luo, Y.; Ni, X.; Zhou, E.M. Molecular detection of spotted fever group rickettsiae in hard ticks, northern China. *Transbound. Emerg. Dis.* **2019**, *66*, 1587–1596. [\[CrossRef\]](#)
- Simser, J.A.; Palmer, A.T.; Fingerle, V.; Wilske, B.; Kurtti, T.J.; Munderloh, U.G. *Rickettsia monacensis* sp. nov., a spotted fever group Rickettsia, from ticks (*Ixodes ricinus*) collected in a European city park. *Appl. Environ. Microbiol.* **2002**, *68*, 4559–4566. [\[CrossRef\]](#)
- Springer, A.; Montenegro, V.M.; Schicht, S.; Wölfel, S.; Schaper, S.R.; Chitimia-Dobler, L.; Siebert, S.; Strube, C. Detection of *Rickettsia monacensis* and *Rickettsia amblyommatis* in ticks collected from dogs in Costa Rica and Nicaragua. *Ticks Tick Borne Dis.* **2018**, *9*, 1565–1572. [\[CrossRef\]](#)
- Ye, X.; Sun, Y.; Ju, W.; Wang, X.; Cao, W.; Wu, M. Vector competence of the tick *Ixodes sinensis* (Acari: Ixodidae) for *Rickettsia monacensis*. *Parasit. Vectors.* **2014**, *7*, 512. [\[CrossRef\]](#)

26. Selmi, R.; Ben Said, M.; Ben Yahia, H.; Abdelaali, H.; Messadi, L. Molecular epidemiology and phylogeny of spotted fever group *Rickettsia* in camels (*Camelus dromedarius*) and their infesting ticks from Tunisia. *Transbound Emerg. Dis.* **2020**, *67*, 733–744. [[CrossRef](#)]
27. Matei, I.A.; Corduneanu, A.; Sándor, A.D.; Ionică, A.M.; Panait, L.; Kalmár, Z.; Ivan, T.; Papuc, I.; Bouari, C.; Fit, N.; et al. *Rickettsia* spp. in bats of Romania: High prevalence of *Rickettsia monacensis* in two insectivorous bat species. *Parasit. Vectors.* **2021**, *14*, 107. [[CrossRef](#)]
28. Lu, M.; Li, F.; Liao, Y.; Shen, J.J.; Xu, J.M.; Chen, Y.Z.; Li, J.H.; Holmes, E.C.; Zhang, Y.Z. Epidemiology and diversity of Rickettsiales bacteria in humans and animals in Jiangsu and Jiangxi provinces, China. *Sci. Rep.* **2019**, *9*, 13176. [[CrossRef](#)]
29. Guo, W.P.; Tian, J.H.; Lin, X.D.; Ni, X.B.; Chen, X.P.; Liao, Y.; Yang, S.Y.; Dumler, J.S.; Holmes, E.C.; Zhang, Y.Z. Extensive genetic diversity of Rickettsiales bacteria in multiple mosquito species. *Sci. Rep.* **2016**, *6*, 38770. [[CrossRef](#)]
30. Matsuura, Y.; Kikuchi, Y.; Meng, X.Y.; Koga, R.; Fukatsu, T. Novel clade of alphaproteobacterial endosymbionts associated with stinkbugs and other arthropods. *Appl. Environ. Microbiol.* **2012**, *78*, 4149–4156. [[CrossRef](#)]