# Prevalence and Phylogenetic Analysis of *Babesia* Parasites in Reservoir Host Species in Fujian Province, Southeast China

**Zhiwei Zeng**
Fujian Center for Disease Control and Prevention https://orcid.org/0000-0002-6622-1370
**Shuheng Zhou**
Fujian Center for Disease Control and Prevention
**Guoying Xu**
Fujian Center for Disease Control and Prevention
**Weijun Liu**
Fujian Center for Disease Control and Prevention
**Tengwei Han**
Fujian Center for Disease Control and Prevention
**Jing Liu**
Fujian Center for Disease Control and Prevention
**Jiaxiong Wang**
Fujian Center for Disease Control and Prevention
**Yanqin Deng**
Fujian Center for Disease Control and Prevention
**Fangzhen Xiao** ( 18642028@qq.com )
Fujian Center for Disease Control and Prevention https://orcid.org/0000-0001-9643-8602

**Research Article**

**Keywords:** Babesia, Mammals, Risk factor, Phylogenetic tree, Southeast China, Babesiosis

**Posted Date:** November 30th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-1110409/v1

# Abstract

**Background:** Babesiosis is a tick-borne disease that mainly affects small mammals and it has been reported in at least five provinces in China. However, the host range and geographical distribution of the parasite in Fujian Province are unclear. Therefore, this study aimed to investigate the prevalence and genetic characteristics of *Babesia* in Fujian Province, Southeast China, between 2015 and 2020.

**Methods:** Rodent blood samples were collected from 26 different surveillance sites across Fujian Province. Genomic DNA was extracted to screen for *Babesia* infection using polymerase chain reaction (PCR) amplification, based on 18S rRNA. DNA samples from 316 domestic goats, 85 water buffalo, 56 domestic dogs, and 18 domestic pigs were examined. The prevalence of *Babesia* was statistically analyzed using the Chi-square test or Fisher's exact test. A phylogenetic tree was constructed using MEGA 5.0 by gene sequence alignment.

**Results:** *Babesia* infections were found in 3.96% (43/1,087) of rodents and 1.26% (6/475) of other mammals. Multivariate logistic regression analysis revealed that irrigated cropland, shrubs, and forests were risk factors for *Babesia microti* infections. The infection rates among domestic pigs, dogs, and goats were found to be 5.56%, 1.79%, and 1.27%, respectively, and no infection was found in water buffalo. Sequencing (18S rRNA gene) revealed that rodents were infected with *Babesia* (sensu lato) while other mammals were infected with *Babesia* (sensu stricto).

**Conclusions:** The results indicate that there is wide geographical distribution and phylogenetic relationship of *Babesia* in Southeast China. This study suggests that mammals, especially wild rodents, are the main natural hosts of *Babesia* in Fujian. Our findings provide a solid foundation for public health officials to develop *Babesia* prevention and control measures.


# Background

Human babesiosis is an emerging, tick-borne zoonotic disease globally [1–3]. It poses a serious threat to public health with global economic, veterinary, and medical significance [1, 4, 5]. Babesiosis is caused by intraerythrocytic sporozoites of the genus *Babesia*, which infect animals (wild and domestic) and humans [5, 6].

The first human case was reported in 1957 in Zagreb, Croatia, and subsequently found on all continents, except for Antarctica. Infections are primarily found in tropical and subtropical areas [7, 8]. More than 60 cases of human babesiosis have recently been reported in China including 48 patients infected with *Babesia venatorum* in Heilongjiang [9], 12 infected with *Babesia divergens* in Shandong [10] and Gansu [7], eight infected with *Babesia microti* in Yunnan [11], and one case infected with *Babesia* sp. XXB/Hangzhou in Zhejiang [12]. During the past few decades, an increasing number of *B. microti* species have been reported in the upper midwestern and northeastern regions of the USA [2, 13]. In Europe, *B. divergens* is responsible for most cases of babesiosis and the parasites are transmitted by *Ixodes ricinus* ticks and infected cows [14, 15]. Endemic infections of *B. microti* in rodents and ticks have been recently

detected in many European countries, including Slovakia [16], Finland [17], Belgium [18], Switzerland [19], Poland [20, 21], and France [22]. Cases have also been recorded in South Africa [23], India [24], and Australia [25]. Therefore, the recent emergence of babesiosis has become a worldwide public health concern.

*Babesia* parasites have a wide range of vertebrate hosts including rodents, horses, goats, cattle, dogs, cats, and humans [5]. More than 100 different *Babesia* species have been discovered; however, only a few are capable of infecting humans, including *Babesia microti* [26], *B. divergens* [7], *B. venatorum* [27], and *Babesia duncani* [28]. As the main etiological agent of human babesiosis, the rodent parasite *B. microti* is maintained through an enzootic cycle in nature, which involves ixodid ticks and small mammals [14, 29]. The clinical characterization of babesiosis ranges from asymptomatic infection to severe morbidity (fever, chills, headache, fatigue, anemia, jaundice, thrombocytopenia, hemolysis, hemoglobinuria, and even multiple organ dysfunction syndromes [MODS]) and may result in death [2]. Susceptibility to *Babesia* infection is usually related to the age and immune status of the host. Neonates, people of advanced age, those undergoing immunosuppressive therapy, and individuals with acquired immune deficiency syndrome (AIDS), or cancer are more susceptible to infection [15, 30]. Babesiosis is frequently overlooked in China, due to a lack of medical awareness, effective diagnostic techniques, and the low incidence of the disease [31, 32]. To date, *B. microti*-like organisms have been reported in humans from Taiwan [33] and Yunnan [34], and *B. microti*-like parasites have been found in small mammals and hard ticks in Yunnan [35], Beijing [3], Taiwan [29], Heilongjiang [36], and Henan [37].

Fujian Province is located on the southeast coast of China, has a subtropical climate, and encompasses 124,000 square kilometers of land and 136,000 square kilometers of ocean. The natural and geographical environments in Fujian provide an ideal habitat for *Babesia* and favorable conditions for the spread of tick-borne diseases, with an average annual rainfall of 1400–2000 mm, abundant sunshine, and 65.95% forest coverage. This study aimed to investigate the infection prevalence and phylogenetic relationship of *Babesia* in mammals across eight cities of Fujian Province, where the host species are abundant.

# Methods

### Sample collection

A total of 1,087 rodents were captured from eight cities in Fujian Province between 2015 and 2020 with live animal traps. The sampling sites included four different habitats: residential areas, irrigated cropland, shrubland, and forests. Live traps were placed every night at every surveillance point for three consecutive nights at locations where rodent activities were detected and were retrieved the following morning. Chinese monographs were used to identify the species of trapped rodents according to their morphology [38, 39]. The sex, age class, and ecological habitat of the mammals were recorded. Any rare rodent species captured were identified using DNA barcoding technology [40]. All animal experiments were performed following the Guidelines for the Care and Use of Laboratory Animals [41]. For anesthesia, the

rodents were placed in a biological safety cabinet, in a transparent plastic box together with cotton wool soaked in ether. After the rodents were anesthetized and disinfected, 1 mL of blood from each rodent was collected through cardiac puncture and stored at –80°C for further tests. Blood samples (2 mL) from 316 domestic goats, 85 water buffalo, 56 domestic dogs, and 18 domestic pigs in Fujian Province were collected, and animals were returned to their farms. Both groups of animals used in this study were part of larger surveillance projects investigating hemorrhagic fever with renal syndrome (rodents) and severe fever with thrombocytopenic syndrome (domestic animals).

### DNA extraction

The Blood Genomic DNA Kit (Tagene Biotechnology, Xiamen, China) was used to extract genomic DNA from the animal blood samples according to the manufacturer's instructions. The genomic DNA was dissolved in 100 μL elution buffer and the 260/280 absorbance ratio was measured to confirm that the DNA purity was between 1.7 and 1.9 using a spectrophotometer (Denovix, DS-11, USA). Samples were stored at –20 ℃ until further use.

### Detection of Babesia infection using polymerase chain reaction

Polymerase chain reaction (PCR) amplification of the specific fragment of the *Babesia* 18S rRNA gene region was performed using the following primers: PIRO–A, 5'–AATACCCAATCCTGACACAGGG–3', PIRO–B, 5'–TTAAATACGAATGCCCCCAAC–3' which were used in previous studies [42, 43]. Target DNA amplification was carried out under the following conditions: 94°C for 5 min; 40 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 45 s; followed by a final extension step at 72°C for 5 min. A concentration of 20–50 ng/μL of genomic DNA (5 μL) was used as a template in a 25 μL reaction, which contained 12.5 μL Premix Taq (TaKaRa Taq Version 2.0 plus dye, Beijing, China) and 1.0 μL of each primer (final concentration 0.4 μM). To eliminate the possibility of contamination, negative (nuclease-free water) and positive controls (a confirmed *Babesia* sample from the first human babesiosis case in Fujian Province) were also included. Amplified products were subjected to electrophoresis on a 1.5% agarose gel stained with SYBR gold (Invitrogen, Shanghai, China) and visualized under ultraviolet light. Positive PCR products were purified and sequenced using the primers listed above, by Sangon Biotechnology Company (Shanghai, China) using Sanger sequencing.

### Phylogenetic analysis

The sequences were assembled using the SeqMan program software 7.0.1 (DNASTAR, Inc.; Madison, WI, USA). All newly generated sequences were subjected to the Basic Local Alignment Tool (BLAST) analysis against the GenBank database. Phylogenetic trees were constructed using the neighbor-joining method, with 1,000 replications for bootstrap tests. All other parameters were set at default.

### Statistical analysis

A spatial map of the prevalence of *Babesia* in rodents was drawn using ArcGIS 10.3.1. The geographic data used the vector map of the administrative divisions of the county boundaries of Fujian Province

(1:1,000,000), and the latitude and longitude were retrieved from Google Maps. The association between rodent species, sex, age class, habitat environments, sampling locations, and *Babesia* infection were analyzed using the univariate analysis based on the Chi-square ($\chi^2$) test or Fisher's exact test. Multivariate logistic regression was used to analyze the risk factors for *B. microti* infection. All analyses were conducted using the SPSS software (version 20.0, SPSS Inc. Chicago, IL, USA). The significance level for all results was set at $P < 0.05$.

**Sequences used for phylogenetic analysis in the study**

To clarify the phylogenetic relationship of *Babesia* species detected in reservoir host species collected in Fujian Province, sequences of the 18S rRNA gene fragments were used for alignment and comparison to the sequences from the GenBank database and are summarized in the additional file 1: Table S1, with additional sequence details if available (species, strains, hosts, years, countries, regions, and GenBank accession numbers).

# Results

**Prevalence of** B. microti **in rodents captured from different cities in Fujian Province**

A total of 1,087 rodents were captured from 26 surveillance points in eight cities in Fujian Province (Fig. 1, Table 1, Additional file 2: Table S2). The captured rodents belonged to the order Rodentia, and they were part of two families, seven genera, and 12 species (Table 2). Sequencing analysis using BLASTn revealed that 3.96% (43/1,087) of the rodents were infected by *B. microti* (Table 1).

Table 1
Prevalence of *B. microti* in rodents in Fujian Province

| Cities | No. of traps | No. of rodents tested | No. of positive samples for *B. microti* | Density (%) | Positive rate (%) | Odds ratio |
|---|---|---|---|---|---|---|
| Sanming | 2,307 | 171 | 17 | 7.41 | 9.94 | 5.96 |
| Ningde | 2,549 | 209 | 15 | 8.20 | 7.18 | 4.18 |
| Nanping | 3,922 | 330 | 8 | 8.41 | 2.42 | 1.34 |
| Fuzhou | 1,790 | 165 | 3 | 9.22 | 1.82 | 1.00 |
| Putian | 355 | 30 | 0 | 8.45 | 0.00 | |
| Quanzhou | 1,549 | 110 | 0 | 7.10 | 0.00 | |
| Zhangzhou | 439 | 41 | 0 | 9.34 | 0.00 | |
| Longyan | 584 | 31 | 0 | 5.31 | 0.00 | |
| Total | 13,495 | 1,087 | 43 | 8.05 | 3.96 | |

Table 2
Prevalence of *Babesia* in different reservoir host species

| Orders | Families | Genera | Species | No. of examined animals | No. of positive | Positive rate (%) |
|---|---|---|---|---|---|---|
| Rodentia | Muridae | *Rattus* | *Rattus norvegicus* | 337 | 3 | 0.89 |
| | | | *Rattus losea* | 215 | 3 | 1.40 |
| | | | *Rattus tanezumi* | 163 | 1 | 0.61 |
| | | | *Rattus edwardsi* | 17 | 1 | 5.88 |
| | | | *Rattus* | 4 | 0 | 0.00 |
| | | *Apodemus* | *Apodemus agrarius* | 32 | 1 | 3.13 |
| | | *Mus* | *Mus musculus* | 5 | 1 | 20.00 |
| | | *Niviventer* | *Niviventer confucianus* | 47 | 8 | 17.02 |
| | | | *Niviventer fulvescens* | 152 | 7 | 4.61 |
| | | *Berylmys* | *Berylmys bowersi* | 50 | 1 | 2.00 |
| | | *Bandicota* | *Bandicota indica* | 56 | 17 | 30.36 |
| | Cricetidae | *Microtus* | *Microtus fortis* | 9 | 0 | 0.00 |
| Artiodactyla | Bovidae | *Capra* | *Capra aegagrus hircus* | 316 | 4 | 1.27 |
| | | *Bos* | *Bos bubalis* | 85 | 0 | 0.00 |
| | Suidae | *Sus* | *Sus scrofa domesticus* | 18 | 1 | 5.56 |
| Carnivora | Canidae | *Canis* | *Canis lupus familiaris* | 56 | 1 | 1.79 |

Of the 12 species of trapped rodents, the brown rat (*Rattus norvegicus*) accounted for the most infections (30.00%, n = 337), followed by *Rattus losea* (19.78%, n = 215), while *Rattus* accounted for the least infections (0.37%, n = 4). Except for *Microtus fortis* and *R. rattus*, 10 of the 12 species tested positive for

*B. microti* infection. The positive infection rates of *B. microti* ranged from 0.61% (1/163) in *Rattus tanezumi* to 30.36% (17/56) in *Bandicota indica* (Table 2). Within the wild rodents (from irrigated cropland, shrubs, and forests), the total infection rate of *B. microti* in the rats *B. indica* and *Niviventer confucianus* was 24.27%, which was significantly higher than that of other rodent species ($\chi^2$ = 66.003, *P* = 0.000).

Infected rodents were captured in four cities: Sanming, Ningde, Nanping, and Fuzhou (Table 1). Rodents collected from Sanming had the highest *B. microti* infection rate of 9.94% (17/171). *Babesia microti* infection rates in the rodents from Sanming and Ningde were both significantly higher than in the rodents captured in Fuzhou (odds ratios: 5.96, 4.18, respectively; *P* < 0.05) (Table 1).

### Prevalence of Babesia in domestic animals in Fujian

The positive infection rates of *Babesia* in domestic pigs, domestic dogs, and domestic goats were 5.56%, 1.79%, and 1.27%, respectively. No water buffaloes were infected with *Babesia* (Table 2). There was no significant difference in the prevalence of *Babesia* between male and female domestic goats (*P* = 0.129) (data not shown).

### Risk factors associated with B. microti infection

Risk factors related to *B. microti* infection in rodents were analyzed with respect to sex, age, and ecological habitat (Table 3). There was no significant difference in the prevalence of *B. microti* between male and female rodents ($\chi^2$ = 0.466, *P* = 0.495). However, the prevalence of *B. microti* in adult rodents (4.53%) was significantly higher ($\chi^2$ = 4.645, *P* = 0.031) than in pubertal rodents (1.10%). It is worth noting that the prevalence of *B. microti* in mammals from irrigated cropland, shrubs, and forests, was 4.70%, 11.18%, and 4.55%, respectively, and were all significantly higher than those in rodents from residential areas (*P* < 0.05, Tables 3 and 4). Furthermore, the multivariate logistic regression analysis suggested that irrigated cropland, shrubs, and forests were risk factors for *B. microti* infection (Table 4).

Table 3
Risk factors related to *Babesia microti* in rodents based on univariate analyses

| Variable | Sample size | | *Babesia microti* infection | | |
|---|---|---|---|---|---|
| | Cases | Constituent ratio (%) | Positive rate (%) | $\chi^2$ | P–value |
| **Sex** | | | | | |
| Male | 561 | 51.61 | 3.57 | 0.466 | 0.495 |
| Female | 526 | 48.39 | 4.37 | | |
| **Age** | | | | | |
| Pubertal | 181 | 16.65 | 1.10 | 4.645 | 0.031 |
| Adult | 906 | 83.35 | 4.53 | | |
| **Habitat** | | | | | |
| Residential Areas | 504 | 46.36 | 0.99 | 35.438 | 0.000 |
| Irrigated Cropland | 149 | 13.71 | 4.70 | | |
| Shrub | 170 | 15.64 | 11.18 | | |
| Forest | 264 | 24.29 | 4.55 | | |

Table 4
Risk factors related to *Babesia microti* infection based on multivariate logistic regression

| Variable | Odds ratio (95% CI) | *P*–value |
|---|---|---|
| **Sex** | | |
| Male | 1 | |
| Female | 0.728 (0.390–1.360) | 0.319 |
| **Age** | | |
| Pubertal | 1 | |
| Adult | 0.307 (0.072–1.304) | 0.110 |
| **Habitat** | | |
| Residential Areas | 1 | |
| Irrigated Cropland | 0.198 (0.061–0.635) | 0.006 |
| Shrub | 0.084 (0.031–0.231) | 0.000 |
| Forest | 0.200 (0.070–0.576) | 0.003 |

### Genetic and phylogenetic analysis of Babesia species

Gene sequencing of the 18S rRNA gene from the positive samples detected 43 samples containing *B. microti*, five containing *Babesia* spp., and one containing *Babesia canis vogeli*. To construct the phylogenetic tree, the 18S rRNA gene sequences of another 18 isolates *of B. microti* from other regions were included for comparison. *Babesia venatorum* from Heilongjiang, *Babesia* sp. XXB/Hangzhou from Zhejiang and *B. divergens* from Ireland were used as the outgroup. All *B. microti* sequences from infected rodents shared 100% homology with sequences from Japan (AB032434.1). The sequence was deposited in GenBank with accession number MZ619064. Phylogenetic analysis revealed that MZ619064 belonged to Kobe-type (Fig. 2).

The sequences of 18S rRNA genes with different *Babesia* species were used to reveal the phylogenetic relationship of *Babesia* identified in this study. *Toxoplasma gondii* (L24381.1) from Australia was used as an outgroup. *Babesia canis vogeli* detected in *Canis lupus familiaris* was identical to the sequences from the Côte d'Ivoire (MK495837.1) and Brazil (KU662365.1). Both domestic pigs and domestic goats in Fujian were infected with *Babesia* sp., and their homology was 98.17%. The sequences of *B. canis vogeli* from *C. lupus familiaris*, *Babesia* sp. from *Sus scrofa domesticus*, and *Babesia* sp. from *Capra aegagrus hircus* in this survey were deposited in GenBank with accession numbers MZ618690, MZ619045, and MZ619046, respectively. Phylogenetic analyses suggested that MZ618690, MZ619045, and MZ619046 belonged to *Babesia* (sensu stricto), while MZ619064 belonged to *Babesia* (sensu lato) (Fig. 3).

# Discussion

Our research systematically illustrated the wide prevalence and phylogenetic relationship of *Babesia* in reservoir host species in Fujian Province, Southeast China. Infections of *B. microti* parasites were observed in four cities and eight sampling sites in Fujian Province (Fig. 1, Table 1). *Babesia microti* has been reported in small mammals in Beijing [3], Henan [37], Yunnan [35], and Taiwan [29]. *Babesia microti* infections was also previously reported in the Wuyi Mountain area, Fujian [44]; however, the epidemiological features of *Babesia* remain unclear in the other cities in Fujian. In this study, the prevalence of *B. microti* in rodents (3.96%) followed the low prevalence described in Yunnan (4.31% [31] and 2.40% [35]), and the Dapan Mountains of Zhejiang (1.30%) [45]. However, the positive infection rates of *B. microti* in *R. tanezumi* in Yunnan (2.70%) and Dapan Mountains (5.56%) were higher than in our research (0.61%). Our study showed that the high prevalence of *B. microti* in *N. confucianus* in Fujian (17.02%) was similar to that found in the Dapan Mountains of Zhejiang (20.0%). This suggested that *N. confucianus* may be superior reservoir hosts in Southeast China [35]. The high prevalence of *B. microti* infection in rodents in Ningde and Sanming in this survey strongly supports the hypothesis that these surveillance points are major natural foci for human babesiosis. Furthermore, the results call for close monitoring of *B. microti* transmissions in Ningde and Sanming, while the epidemic of *B. microti* in other cities should not be ignored. It should be noted that the *B. microti* infection rates were zero in Putian, Quanzhou, Zhangzhou, and Longyan, which may be attributed to a lack of samples and rodent habitats (Table 4). The prevalence of *B. microti* varied from district to district. Although both Fuzhou and Quanzhou are adjacent to Sanming, the *B. microti* infection rates were lower than 5.00% for both, while Sanming had the highest infection rate. This may be attributed to the distribution and density of the reservoir host species.

Interestingly, the infection rate of *Babesia* in Xiapu District, Ningde City, was 15.79%, which may provide a novel link to the first human case of babesiosis in Fujian [46]. The patient, who was diagnosed with a *B. microti* infection, lived and worked in a village in Xiapu, Ningde, which was surrounded by abundant shrubs and forests. Our study revealed that the prevalence of *B. microti* in rodents from shrubs (11.18%), irrigated cropland (4.70%), and forests (4.55%) was significantly higher than in the residential areas (0.99%), suggesting that ecological habitat types played an important role in the spread of *B. microti*. Furthermore, *B. microti* would be able to live and reproduce in the wild, which is likely related to the habitat and density of the tick vector [35]. Similar results have been reported in Yunnan [35] and Beijing [3]. The prevalence of *B. microti* in small mammals in Yunnan from the forest (3.37%) and agricultural areas (1.79%) was significantly higher than in residential areas (0.93%). In Beijing, the positive rate of *B. microti* from different habitats was shrubs (27.4%), broad-leaved forests (23%), cropland (16%), mixed forests (8.4%), and residential areas (7.2%). It has been reported that forests are an essential risk factor for *Babesia* infection in Thailand, Cambodia, Lao PDR, and China (Yunnan and Heilongjiang) [32, 35, 36]. Considering that forest areas are burdened with tick-transmitting pathogens, people who work in or travel to forests should take appropriate protective measures. Both *Babesia* and *Plasmodium* are intraerythrocytic protozoans and elicit similar inflammatory responses with similar clinical symptoms, which allows them to be easily misdiagnosed [47]. In summary, doctors should pay attention to human

babesiosis, while public health agencies should urgently formulate prevention and control measures. Our study found that the prevalence of *B. microti* in adult rodents (4.53%) was significantly higher than in pubertal rodents (1.10%), which was similar to other studies in Yunnan (2.69% (adult) and 0.37% (pubertal)) [35] and Beijing (13.3% (adult) and 6.2% (pubertal)) [3].

Our study revealed that all the collected *Babesia* parasites were *B. microti* from rodents in Fujian Province. This conclusion can be drawn from the abundance of samples detected, which was similar to previous findings in Yunnan [31, 35], Taiwan [29], and Beijing [3]. Phylogenetic analysis suggested that *B. microti* in this survey shared high homology with those in Zhejiang Province, where a confirmed human babesiosis case was reported in Hangzhou in 2002 following kidney transplantation [44]. Surprisingly, no water buffaloes were infected with *Babesia bovis* or *Babesia orientalis*. This varied in comparison to other studies [48, 49], which might be due to insufficient samples and a single sampling location. In addition, both domestic goats and domestic pigs were infected with *Babesia spp*. We detected *B. canis vogeli* from the blood of domestic dogs for the first time and the sequence shares high homology with *B. canis vogeli* from Côte d'Ivoire (GenBank MK495837.1). The prevalence of *B. canis* in dogs has been previously documented in Henan province [50].

Ovine babesiosis is a tick-borne disease in goats, sheep, and cattle, posing a huge threat to the livestock industry [51–53]. Although the infection rate of ovine babesiosis is extremely low in this study, relevant institutions should pay more attention and strengthen quarantine measures for early detection and treatment. *Babesia* infection can also be transmitted through blood transfusion when the infected individual is asymptomatic or in the latent period of the infection [30]. Therefore, it is necessary to test *Babesia* infection in donors when evaluating the risk of blood transfusion. In the future, we will investigate the prevalence of *Babesia* in different ticks and blood donors to provide scientific evidence for preventing and controlling babesiosis epidemics.

There are limitations to this study. A nested PCR approach was used to investigate the rates of *Babesia* parasites in mammals in some reports [31, 32]. A large number of samples (n = 1,562) with a high risk of contamination were part of this study, therefore the nested PCR method was not used. Our study showed that the prevalence of *B. microti* was higher in northern Fujian (Sanming, Nanping, Ningde, and Fuzhou) than in southern Fujian (Putian, Quanzhou, Zhangzhou, and Longyan), which could be due to altitude, as northern Fujian (495 meter) has a higher average altitude than southern Fujian (411 meter). A study in southern Norway has shown that ticks exist at an altitude much higher than previously, with an increased risk of infection of mammals with tick-borne diseases [54]. It was reported that altitude is a risk factor associated with *Babesia* infections [3, 35]; however, altitude was not taken into account in this study. With the development of the economy and the improvement of living standards in China, the number of domesticated dogs and cats has increased, resulting in *B. vogeli* cases in dogs and cats [4, 50, 55]. Therefore, it is necessary to monitor *Babesia* infections in domestic dogs and cats.

# Conclusions

Our study suggests a wide distribution and phylogenetic relationship of *Babesia* in mammals in Fujian Province, Southeast China. This research provided basic data to help the public health authorities develop prevention and control measures. Due to insufficient samples from single sampling surveillance, hosts such as livestock should be investigated. Because human babesiosis is a tick-borne disease that is transmitted through blood transfusions, it is necessary to survey the prevalence of *Babesia* in various tick and donor populations.

# Abbreviations

DNA: deoxyribonucleic acid; 18S rRNA: 18 Svedberg ribosomal ribonucleic acid; PCR: polymerase chain reaction; *CI*: confidence interval; MODS: multiple organ dysfunction syndrome; AIDS: acquired immune deficiency syndrome; BLAST: Basic Local Alignment Search Tool.

# Declarations


## Acknowledgements

We would like to thank all the participants for their commitment. We would like to thank Editage (www.editage.cn) for English language editing. We also thank the staff of the Centers for Disease Control and Prevention of Fuzhou District, Putian District, Quanzhou District, Zhangzhou District, Longyan District, Sanming District and Nanping District, Ningde District, Fujian, China for their assistance in collecting samples.

## Funding

This work was sponsored by the National Science and Technology Major Project of China (No. 2018ZX10734401–007), Fujian Provincial Science and Technology Innovation Platform Construction Project (2019Y2001), and Fujian Provincial Health Technology Project (2019–ZQN–27). The funders had no role in the design of the study, collection, analysis, and interpretation of data, or in writing the manuscript.


## Availability of data and materials

The data collected and analyzed during the current study are available from the corresponding author upon reasonable request. Please contact the author for the data requests.

## Authors' contributions

FZX and YQD designed the study and contributed to reviewing the manuscript. ZWZ drafted the manuscript, performed the statistical analysis, and participated in the sample collection. SHZ, WJL, TWH, GYX, JL, and JXW conducted the molecular biological assays, sampling acquisition, and identified the host animal species. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The research protocol, which involved trapping wild and domestic animals, was approved by the Laboratory Animal Welfare Ethical Review Committee of Fujian Provincial Center for Disease Control and Prevention (FJCDC) (permission number: FJCDCNT1811–2015). All animal experiments were performed following the Guidelines for the Care and Use of Laboratory Animals.

### Consent for publication

Not applicable.

### Competing interests

The authors have declared that no competing interests exist.

# References

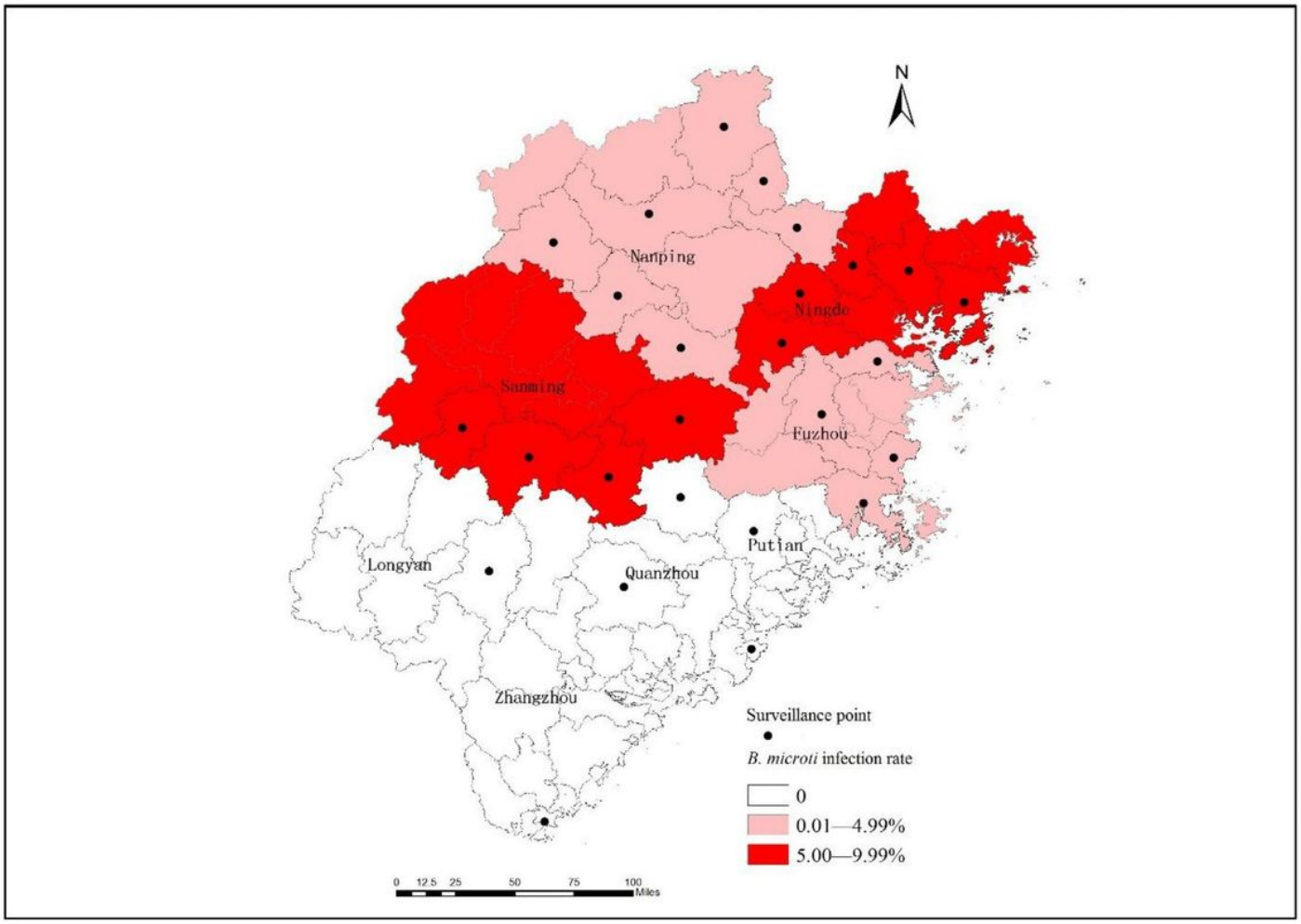

1. Vannier E, Krause PJ. Human babesiosis. N Engl J Med. 2012;366:2397–407.
2. Krause PJ. Human babesiosis. Int J Parasitol. 2019;49:165–74.
3. Wei CY, Wang XM, Wang ZS, Wang ZH, Guan ZZ, Zhang LH, et al. High prevalence of *Babesia microti* in small mammals in Beijing. Infect Dis Poverty. 2020;9:155.
4. Li XW, Zhang XL, Huang HL, Li WJ, Wang SJ, Huang SJ, et al. Prevalence and molecular characterization of *Babesia* in pet dogs in Shenzhen, China. Comp Immunol Microbiol Infect Dis. 2020;70:101452.
5. Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA. *Babesia*: a world emerging. Infect Genet Evol. 2012;12:1788–809.
6. Jalovecka M, Sojka D, Ascencio M, Schnittger L. *Babesia* life cycle – when phylogeny meets biology. Trends Parasitol. 2019;35:356–68.
7. Wang J, Zhang S, Yang J, Liu J, Zhang D, Li Y, et al. *Babesia divergens* in human in Gansu Province, China. Emerg Microbes Infect. 2019;8:959–61.
8. Liu AH, Yin H, Guan GQ, Schnittger L, Liu ZJ, Ma ML, et al. At least two genetically distinct large *Babesia* species infective to sheep and goats in China. Vet Parasitol. 2007;147:246–51.
9. Jiang JF, Zheng YC, Jiang RR, Li H, Huo QB, Jiang BG, et al. Epidemiological, clinical, and laboratory characteristics of 48 cases of "*Babesia venatorum*" infection in China: a descriptive study. Lancet Infect Dis. 2015;15:196–203.
10. Qi C, Zhou D, Liu J, Cheng Z, Zhang L, Wang L, et al. Detection of *Babesia divergens* using molecular methods in anemic patients in Shandong Province, China. Parasitol Res. 2011;109:241–5.
11. Zhou X, Li SG, Chen SB, Wang JZ, Xu B, Zhou HJ, et al. Co-infections with *Babesia microti* and *Plasmodium* parasites along the China-Myanmar border. Infect Dis Poverty. 2013;2:24.

12. Man SQ, Qiao K, Cui J, Feng M, Fu YF, Cheng XJ. A case of human infection with a novel *Babesia* species in China. Infect Dis Poverty. 2016;5:28.
13. Liu HH, Cushinotto L, Giger O, Daum G, McBride P, Negron EA, et al. Increasing babesiosis in Southeastern Pennsylvania, 2008-2017. Open Forum Infect Dis. 2019;6:ofz066.
14. Gray JS. Identity of the causal agents of human babesiosis in Europe. Int J Med Microbiol. 2006;296 Suppl 40:131–6.
15. Martinot M, Zadeh MM, Hansmann Y, Grawey I, Christmann D, Aguillon S, et al. Babesiosis in immunocompetent patients, Europe. Emerg Infect Dis. 2011;17:114–6.
16. Blaňarová L, Stanko M, Miklisová D, Víchová B, Mošanský L, Kraljik J, et al. Presence of *Candidatus Neoehrlichia mikurensis* and *Babesia microti* in rodents and two tick species (*Ixodes ricinus* and *Ixodes trianguliceps*) in Slovakia. Ticks Tick Borne Dis. 2016;7:319–26.
17. Kallio ER, Begon M, Birtles RJ, Bown KJ, Koskela E, Mappes T, et al. First report of *Anaplasma phagocytophilum* and *Babesia microti* in rodents in Finland. Vector Borne Zoonotic Dis. 2014;14:389–93.
18. Lempereur L, De Cat A, Caron Y, Madder M, Claerebout E, Saegerman C, et al. First molecular evidence of potentially zoonotic *Babesia microti* and *Babesia sp.* Vector Borne Zoonotic Dis. 2011;11:125–30.
19. Foppa IM, Krause PJ, Spielman A, Goethert H, Gern L, Brand B, et al. Entomologic and serologic evidence of zoonotic transmission of *Babesia microti*, eastern Switzerland. Emerg Infect Dis. 2002;8:722–6.
20. Siński E, Bajer A, Welc R, Pawełczyk A, Ogrzewalska M, Behnke JM. *Babesia microti*: prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes District of North-Eastern Poland. Int J Med Microbiol. 2006;296;Suppl 40:137–43.
21. Tołkacz K, Bednarska M, Alsarraf M, Dwużnik D, Grzybek M, Welc-Falęciak R, et al. Prevalence, genetic identity and vertical transmission of *Babesia microti* in three naturally infected species of vole, *Microtus* spp. (Cricetidae). Parasit Vectors. 2017;10:66.
22. Jouglin M, Perez G, Butet A, Malandrin L, Bastian S. Low prevalence of zoonotic *Babesia* in small mammals and *Ixodes ricinus* in Brittany, France. Vet Parasitol. 2017;238:58–60.
23. Bush JB, Isaäcson M, Mohamed AS, Potgieter FT, de Waal DT. Human babesiosis: a preliminary report of 2 suspected cases in South Africa. S Afr Med J. 1990;78:699.
24. Marathe A, Tripathi J, Handa V, Date V. Human babesiosis: a case report. Indian J Med Microbiol. 2005;23:267–9.
25. Senanayake SN, Paparini A, Latimer M, Andriolo K, Dasilva AJ, Wilson H, et al. First report of human babesiosis in Australia. Med J Aust. 2012;196:350–2.
26. Goethert HK, Molloy P, Berardi V, Weeks K, Telford SR, 3rd. Zoonotic *Babesia microti* in the northeastern U.S.: evidence for the expansion of a specific parasite lineage. PLoS One. 2018;13:e0193837.

27. Sun Y, Li SG, Jiang JF, Wang X, Zhang Y, Wang H, et al. *Babesia venatorum* Infection in Child, China. Emerg Infect Dis. 2014;20:896–7.
28. Conrad PA, Kjemtrup AM, Carreno RA, Thomford J, Wainwright K, Eberhard M, et al. Description of *Babesia duncani* n.sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. Int J Parasitol. 2006;36:779–89.
29. Chao LL, Yu WC, Shih CM. First detection and molecular identification of *Babesia microti* in *Rattus losea* captured from the offshore Kinmen Island of Taiwan. Ticks Tick Borne Dis. 2017;8:313–9.
30. Bloch EM, Yang Y, He M, Tonnetti L, Liu Y, Wang J, et al. A pilot serosurvey of *Babesia microti* in Chinese blood donors. Vox Sang. 2018;113:345–9.
31. Chen XR, Ye LI, Fan JW, Li C, Tang F, Liu W, et al. Detection of Kobe-type and Otsu-type *Babesia microti* in wild rodents in China's Yunnan Province. Epidemiol Infect. 2017;145:2704–10.
32. Karnchanabanthoeng A, Morand S, Jittapalapong S, Carcy B. *Babesia* occurrence in rodents in relation to landscapes of mainland Southeast Asia. Vector Borne Zoonotic Dis. 2018;18:121–30.
33. Shih CM, Liu LP, Chung WC, Ong SJ, Wang CC. Human babesiosis in Taiwan: asymptomatic infection with a *Babesia microti*-like organism in a Taiwanese woman. J Clin Microbiol. 1997;35:450–4.
34. Zhou X, Li SG, Wang JZ, Huang JL, Zhou HJ, Chen JH, et al. Emergence of human babesiosis along the border of China with Myanmar: detection by PCR and confirmation by sequencing. Emerg Microbes Infect. 2014;3:e55.
35. Gao ZH, Huang TH, Jiang BG, Jia N, Liu ZX, Shao ZT, et al. Wide distribution and genetic diversity of *Babesia microti* in small mammals from Yunnan Province, Southwestern China. PLoS Negl Trop Dis. 2017;11:e0005898.
36. Sun Y, Liu G, Yang L, Xu R, Cao W. *Babesia microti*-like rodent parasites isolated from *Ixodes persulcatus* (Acari: Ixodidae) in Heilongjiang Province, China. Vet Parasitol. 2008;156:333–9.
37. Zhao XG, Li H, Sun Y, Zhang YY, Jiang JF, Liu W, et al. Dual infection with *Anaplasma phagocytophilum* and *Babesia microti* in a *Rattus norvegicus*, China. Ticks Tick Borne Dis. 2013;4:399–402.
38. Huang WJ. Rodents of China. Shanghai: Fudan University Press; 1995.
39. Zhang RZ. Distribution of mammalian species in China. Beijing: China Forestry Publishing House; 1999.
40. Liu WJ, Xu GY, Xiao FZ, Lin DH, Liu J, Han TW. Application of DNA bar coding in identifying rodent species in Fujian Province, China. Chin J Vector Biol Control. 2020;31:175–9.
41. National Research Council (US) Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US); 1996.
42. Stahl P, Poinsignon Y, Pouedras P, Ciubotaru V, Berry L, Emu B, et al. Case report of the patient source of the *Babesia microti* R1 reference strain and implications for travelers. J Travel Med. 2018;25.
43. Olmeda AS, Armstrong PM, Rosenthal BM, Valladares B, del Castillo A, de Armas F, et al. A subtropical case of human babesiosis. Acta Trop. 1997;67:229–34.

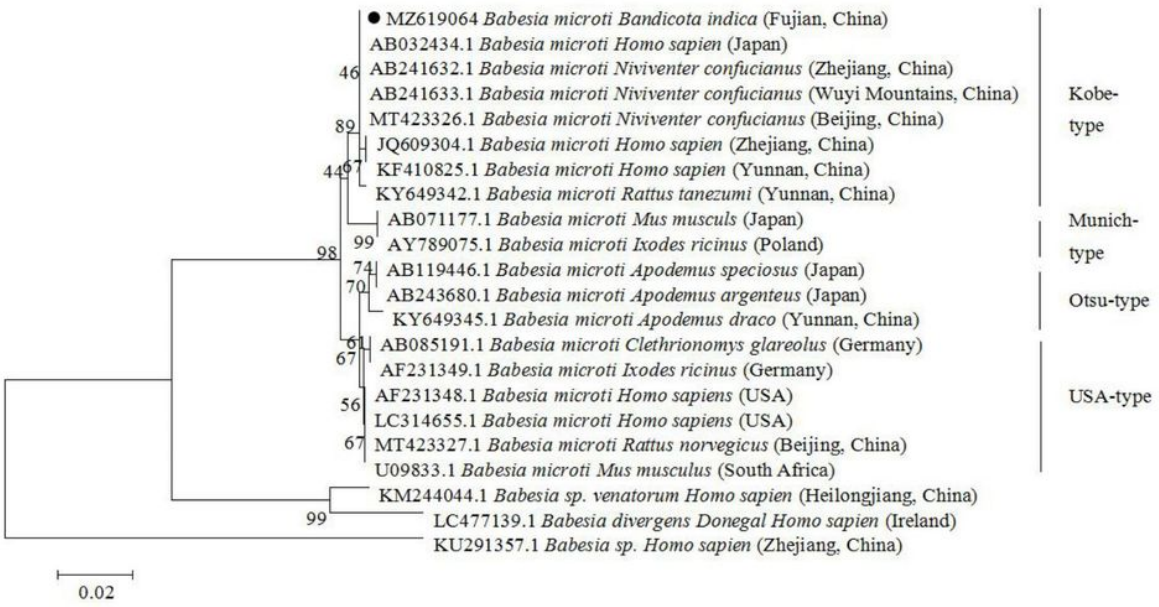
44. Saito-Ito A, Takada N, Ishiguro F, Fujita H, Yano Y, Ma XH, et al. Detection of Kobe-type *Babesia microti* associated with Japanese human babesiosis in field rodents in central Taiwan and southeastern mainland China. Parasitology. 2008;135:691–9.
45. Wei YC, Jiang LP, Ye JL, Ying KM, Zheng BF. Molecular survey of *Babesia* in rodents from Dapan Mountain, Zhejiang. China Prev Med. 2013;14:949–52.
46. Ou YR, Chen ZY, Lin YY, Xiao LZ, Xie HG. Diagnosis and identification for human babesiosis in Fujian Province, China. Chin J Zoonoses. 2018;34:492–4.
47. Zhou X, Xia S, Huang JL, Tambo E, Zhuge HX, Zhou XN. Human babesiosis, an emerging tick-borne disease in the People's Republic of China. Parasit Vectors. 2014;7:509.
48. He L, Liu Q, Yao B, Zhou Y, Hu M, Fang R, et al. A historical overview of research on *Babesia orientalis*, a protozoan parasite infecting water buffalo. Front Microbiol. 2017;8:1323.
49. He L, Feng HH, Zhang WJ, Zhang QL, Fang R, Wang LX, et al. Occurrence of *Theileria* and *Babesia* species in water buffalo (*Bubalus babalis*, Linnaeus, 1758) in the Hubei Province, South China. Vet Parasitol. 2012;186:490–6.
50. Wang J, Liu J, Yang J, Liu Z, Wang X, Li Y, et al. Molecular detection and genetic diversity of *Babesia canis* in pet dogs in Henan Province, China. Parasitol Int. 2019;71:37–40.
51. Niu Q, Liu Z, Yang J, Yu P, Pan Y, Zhai B, et al. Genetic diversity and molecular characterization of *Babesia motasi*-like in small ruminants and ixodid ticks from China. Infect Genet Evol. 2016;41:8-15.
52. Guswanto A, Allamanda P, Mariamah ES, Sodirun S, Wibowo PE, Indrayani L, et al. Molecular and serological detection of bovine babesiosis in Indonesia. Parasit Vectors. 2017;10:550.
53. He L, Bastos RG, Sun Y, Hua G, Guan G, Zhao J, et al. Babesiosis as a potential threat for bovine production in China. Parasit Vectors. 2021;14:460.
54. De Pelsmaeker N, Korslund L, Steifetten Ø. High-elevational occurrence of two tick species, *Ixodes ricinus* and *I. trianguliceps*, at their northern distribution range. Parasit Vectors. 2021;14:161.
55. Zhang XL, Li XW, Li WJ, Huang HL, Huang SJ, Shao JW. Molecular evidence of *Babesia* in pet cats in mainland China. BMC Vet Res. 2019;15:476.

# Figures




## Figure 1

Prevalence of Babesia microti in rodents across 26 surveillance points in eight cities in Fujian Province, Southeast China. The prevalence of Babesia microti infection in each city is indicated by different colors. In the legend at the lower right corner, red indicates high infection rates, while white presents low infection rates. The geographic location of each surveillance point is labeled with black dots on the map




## Figure 2

Neighbor-joining phylogenetic tree based on Babesia microti 18S rRNA partial sequence data from Fujian isolates with Babesia microti reference strains. Babesia divergens, Babesia sp. XXB/Hangzhou and Babesia venatorum were used as the outgroup. For reference, taxon names include the corresponding GenBank accession number, Babesia species, hosts, and regions of isolation. The number on each branch indicates the percent occurrence in 1,000 bootstrap replicates. Black circles represent novel sequences identified in this work

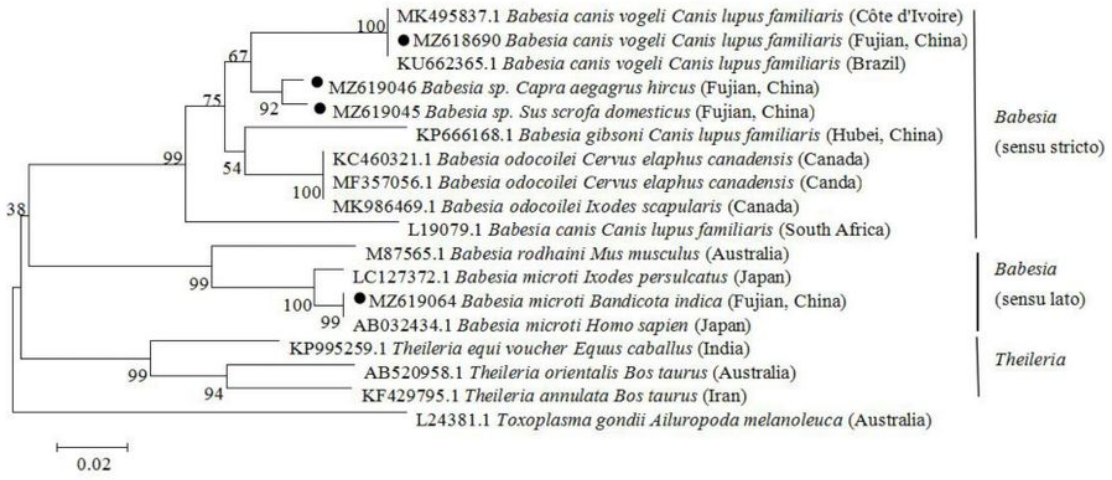



**Figure 3**

Neighbor-joining phylogenetic tree based on Babesia species, 18S rRNA partial sequence data from Fujian isolates with Babesia species reference strains. Toxoplasma gondii (L24381.1) was used as the outgroup. For reference, taxon names include the corresponding GenBank accession number, Babesia species, hosts, and regions of isolation. The number on each branch indicates the percent occurrence in 1,000 bootstrap replicates. Black circles represent novel sequences identified in this work

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile120211124.docx
- Additionalfile220211124.docx
- GraphicalAbstract20211124.pdf