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RESEARCH ARTICLE

Investigation of chigger mites on small mammals in Ruili, Yunnan Province, China

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ABSTRACT

Chigger mites are arthropods and are the sole vectors of scrub typhus, and rodents as well as other small mammals are the most common hosts of chigger mite larvae. Therefore, it is of great medical significance to study the ecology of chigger mites. In this study, a detailed analysis of chigger mites was conducted based on field survey data. A total of 4,941 chigger mites were collected from 86 hosts at 34 survey sites in Ruili, Yunnan Province, China. Among the 4,941 chiggers, five genera in one subfamily were identified; *Schoengastiella ligula* was the dominant chigger species with the highest infestation index, prevalence (*Pm*, 42.86%) and mean intensity (*MI*, 59.09%) (*P<0.001*). The association coefficient (*V)* between *S. ligula* and *Gahrliepia radiopunctata* was positively correlated (*P<0.05*), indicating the tendency of chiggers to select and coexist on the same host at the same time. The dominant species *Leptotrombidium kunmingense, Ascoschoengastia indica*, *S. ligula* and *G. radiopunctata* showed aggregation distribution patterns, indicating that the distribution of chiggers among different hosts was not uniform. Low altitudes and low latitudes appeared to be more favorable for the growth and reproduction of chigger mites (*P<0.05*). It is suggested to collect as many host samples as possible in future field investigations to better understand the dynamics of chigger mite populations and their primary hosts.

Keywords: Chigger mites; scrub typhus; small mammals; Yunnan region.

INTRODUCTION

Chigger mites are complex arthropods with a seven-stage life cycle, but only the larvae are ectoparasitic, and rodents, as well as other small mammals, are the most common hosts for chigger larvae (Chen *et al*., 2021; Ding *et al*., 2021a). Globally, 3,700 species of chigger mites have been identified, and among them, 60 are capable of transmitting scrub typhus. In China, 531 species of chigger mites have been identified, of which six transmit scrub typhus (including *Leptotrombidium deliense*, *L. scutellare*, *L. rubellum*, *L. sialkotense*, *L. wenense* and *L. insulare*). A total of 274 species of chigger mites have been identified in Yunnan, five of which transmit scrub typhus (*Leptotrombidium deliense*, *L. scutellare*, *L. rubellum*, *L. wenense* and *L. insulare*) (Lv *et al*., 2018; Xiang & Guo, 2021; Chen *et al*., 2022). Chigger mites are thought to be the only vector of scrub typhus; however, in addition to the transmission of tsutsugamushi, chigger mites are suspected of transmitting the virus that causes hemorrhagic fever in China (Lee *et al*., 2009; Peng *et al*., 2016a; Liu & Ren, 2022). Scrub typhus, caused by the pathogen *Orientia tsutsugamushi*, is the second most common febrile disease after malaria and is transmitted to humans through the bite of an infected chigger mite (chigger mite larvae) (Lv *et al*., 2018; Ding *et al*., 2021b; Lee *et al*., 2021). The "scrub typhus triangle" is a densely populated region containing more than 1 billion people at risk of infection, and approximately 1 million new cases are reported each year, with a mortality rate of 30% or higher if not properly treated at the early stage (Xu et *al*., 2017; Lv *et al*., 2018, 2021). According to previous a study, more than half (55%) of the world's population now lives in places where scrub typhus is prevalent (Yao *et al*., 2019). However, there is still no effective and reliable vaccine against scrub typhus,and an immediate diagnosis is challenging; thus, scrub typhus poses a major health risk to the public in endemic areas (Xu *et al*., 2017).

In recent years, the incidence of scrub typhus has increased in China and has caused nonnegligible burdens on public health resources and the economy. The latest surveillance data indicated that scrub typhus has spread to all parts of China except Ningxia and Shanghai, and approximately 162 million people in southern China are at risk of infection; Guangdong and Yunnan had the highest rates of scrub typhus (Yue *et al*., 2019; Li *et al*., 2021; Peng *et al*., 2022). During 2006-2017, 30,001 cases of scrub typhus were reported in southwest China (including Sichuan and Yunnan provinces); among them, 26,898 (89.7%) were reported in Yunnan Province. A total of 29,121 (97.1%) were suspected cases, and 880 (2.9%) were confirmed cases, In Yunnan Province, the number of cases was 20.64 times higher in 2017 than in 2006, with the highest incidence rates in Baoshan, Lincang and Dehong Prefecture (Xin *et al*., 2020; Peng *et al*., 2022). Yunnan is known as the "Kingdom of

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Flora and Fauna", because of its complex geological conditions and rich biodiversity, and it contains many national cross-border nature reserves in southeast China (Shen *et al*., 2017). This rich biodiversity may account for the high host species diversity associated with scrub typhus in Yunnan Province, indirectly contributing to a large number of scrub typhus cases (Peng *et al*., 2022). Therefore, studying the ecology of chiggers in Yunnan Province is important because it will allow deeper insights into the dynamics of chigger populations and major hosts (Zhan *et al*., 2013). The survey site (Ruili) in the current study is located in Dehong Prefecture, the epidemiological focus of the scrub typhus epidemic. A series of field surveys of ectoparasites on rodent and small mammal hosts within Ruili was conducted in 2020, and the present study reports the results of these surveys and describes the detailed investigation of the community structure, spatial distribution, interspecific relationships, phylogeographic clustering and associated risk factors for chigger mite infection.

MATERIALS AND METHODS

Ethical statement

The capture and use (including euthanasia) of rats and small mammals in this study was approved by the Wildlife Administration of Yunnan Province, China, and the Animal Ethics Committee of Dali University under animal ethics permission number MECDU-201806-11.

Field investigation

Yunnan Province is an inland province located in a border area in southwest China. It has a low latitude and high altitude and is located between 21°82 to 29°152 N and 97°312 to 106°112 E. The geographical landscape is complex, and the flora and fauna biodiversity is rich and unique. Ruili, Dehong Prefecture, is located on the northern side of the Tropic of Cancer. The climate is mild throughout the year, with an annual average temperature of 21.0°C. The average temperatures are 14.0°C in the coldest month and 25.0°C in the hottest month. The annual average rainfall is 1384.5 mm, and the annual average relative humidity is 76% (http://www.rl.gov.cn/Web/_F0_0_4QWG188R77D6913878FD4B7E96.html), the natural geographical conditions are unique and provide adequate conditions for the survival and reproduction of chiggers and their hosts. Field surveys were conducted in Ruili, Dehong Prefecture, western Yunnan Province, for three consecutive months from August 2020 to October 2020, the sampling period in each month was lasting 10 for days.

Collection and identification of hosts and chigger mites

Random sampling was conducted at various longitudes, latitudes and altitudinal gradients, and in different ecosystems. Traps were set at each survey site at 17:00-18:00 and checked in the next morning between 07:00-08:00, with captured animal hosts placed individually in premarked white cloth bags and then transferred to a temporary laboratory where they were anesthetized with ether, Small mammals were identified based on characteristics such as fur color and external morphology, chiggers were collected from each host and preserved in 70% ethanol vials to make slide specimens, and all chigger specimens were eventually identified by microscopy (Guo *et al*., 2013; Peng *et al*., 2016b; Chen *et al*., 2022). Representative animal hosts and chigger mite specimens were deposited in the Herbarium of the Institute of Pathogens and Vectors, Dali University, Yunnan Province, China.

Chigger mite infestation statistics

The constituent ratio (*Cr*), prevalence (*Pm*), mean abundance (*MA*), and mean intensity (*MI*) of surface chigger mites were used to calculate composition ratios and determine the infection status of animal hosts. All chiggers on the surface of each host were considered a community unit, and mite community structure was described using abundance (*S*), the Shannon–Wiener diversity index (*H'*), community evenness (*J'*) and Simpson's dominance index (*D*). In this study, the infection rate (*Pm*) was statistically determined using the chi-square test (χ^2), and mean multiplicity (*MA*) and intensity (*MI*) were analyzed for significance using the Mann–Whitney test or Kruskal–Wallis test (Zhang *et al*., 2009; Ding *et al*., 2021b; Chen *et al*., 2022).

$$C_r = \frac{\mathrm{Ni}}{N}\times 100\,\%;\ P_m = \frac{H\mathrm{i}}{H}\times 100\,\%;\ MA = \frac{\mathrm{Ni}}{H};\ MI = \frac{\mathrm{Ni}}{Hi},$$

$$S = \sum S_i;\ H' = -\sum_{i=1}^{s}\left(\frac{Ni}{N}\right)\ln\left(\frac{Ni}{N}\right);\ J' = \frac{H'}{\ln S};\ D = \sum_{i=1}^{s}\left(\frac{Ni}{N}\right)^2,$$

In the above equation, *Ni* = individuals of the i-th species of chiggers on the host; *N* = total individuals of all chiggers on the host, *Hi* = individuals of the infected host and *H* = total individuals of the host; *S* = number of species in the community, *Si* = i-th species in the community, *Ni* is the number of individuals of the i-th species, and *N* is the cumulative number of individuals of all species in the community (total number of individuals).

The number of individuals, composition ratio, species richness, community evenness and diversity index of the host were used as indicators to construct the original matrix. SPSS 26.0 software was used for computations, and the nearest-neighbor clustering method was applied. Pearson correlation analysis was performed, and Z scores were calculated for the standardization of variables; the correlation clustering analysis results are shown in a tree diagram (Zhang *et al*., 2009; Peng, 2019).

Determination of the spatial distribution pattern of chigger mites

The spatial distributions of chiggers among different host individuals were analyzed, and the dispersion coefficient (*C*), patchiness index (*m*/m*), I-index (*I*) and Cassie index (*CA*) were calculated to determine the spatial patterns of dominant species (Guo *et al*., 2006, 2013; Ding *et al*., 2020).

$$C = \frac{\sigma^2}{m};\ C_A = \frac{\sigma^2 - m}{m^2};\ I = C - 1;\ {}^{m*}/_{m} = \frac{m + I}{m}$$

In the above equation, m = mean number of chiggers on each host, σ^2 = variance in chiggers on each host, and *m** = mean crowding of chiggers on each host. The judgment criteria were as follows: if *m*/m* >1, the spatial pattern was determined to be aggregated; if *m*/m* =1, there was a random distribution, and if *m*/m* <1, there was a uniform distribution.

Analysis of interspecific relationships

The association coefficient (*V*) was used to determine the interspecific relationship among the main chigger species on the body surface of the dominant host. The chi-square test was used to assess the statistical significance of *V* (Yin *et al*., 2021).

$$V = \frac{ad - bc}{\sqrt{(a+\mathrm{b})(\mathrm{c}+d)(a+c)(b+d)}},$$

In the above equation, *V* = association coefficient; *a* = number of hosts on which both species x and species y occur; *b* = number of hosts on which species y occurs but species x does not; *c* = number of hosts on which species x occurs but species y does not; and *d* = number of hosts on which neither species x nor species y occurs. The judgment criteria were as follows: there was a positive correlation when $V > 0$, $P<0.05$; a negative correlation when $V < 0$, $P<0.05$; and no correlation when $V = 0$. *P* is the probability of significance in the chi-square test (χ^2).

Host selection and distribution analyses

The Sorensen index (*Css*) was used to determine the species compositions of chigger mites on different hosts. To reflect the horizontal distribution of mites, host infestations were compared according to latitude and longitude, which were divided into two latitude strata, namely, < 24°N and 24°N-25°N; and two longitude strata, namely < 98°E and 98°E-99°E. To reflect the vertical distribution of mites, host infestations were compared according to altitude, which was divided into four strata: < 800 m, 800-1000 m and > 1000 m (Peng *et al*., 2015; Liu *et al*., 2019; Lv, 2021).

$$C_{ss} = \frac{2C}{S_m + S_f},$$

In the above equation, S_m = number of chigger mite species on male hosts; S_f = number of chigger mite species on female hosts; and C = number of chigger mite species on both male and female hosts.

RESULTS

Collection and identification of chigger mites

Among 182 rodents examined at 34 survey sites in Ruili (Table 1) (Figure 1), 86 were infected with chiggers, and a total of 4,941 chiggers belonging to one subfamily and five genera were collected and identified (Table 2). Six species (*Leptotrombidium kunmingense*, *Microtrombicula munda*, *Ascoschoengastia indica*, *Schoengastiella ligula*, *Schoengastiella punctata* and *Gahrliepia radiopunctata*) of chiggers were isolated and identified from hosts (*Rattus tanezumi*, *Suncus murinus*, *Rattus brunneusculus*, *Bandicota indica*, *Rattus nitidus* and *Niviventer confucianus*), with an overall *Pm* of 47.25% and an overall *MI* of 57.45. *Schoengastiella ligula* was the dominant chigger mite species, with the highest *Pm* of 42.86% and *MI* of 59.09 (P<0.001), followed by *L. kunmingense* (*Pm* = 42.86% and *MI* = 59.09, P<0.01) (Table 3). Among the six species (4941 individuals) of chiggers identified, the community structure of chigger mites on hosts was as follows: S = 6, H' = 0.67, J' = 0.37 and D = 0.62. Chigger mite richness was highest on *R. tanezumi*, which harbored six species, followed by *B. indica*, which harbored four species; the diversity index was highest for *R. tanezumi*, at 0.79, followed by *B. indica*, at 0.014 (Table 4).

In the cluster diagram of parasitic mite communities on small-animal hosts, the communities are clustered in order of similarity, with the similarity between the communities decreasing as clustering progressed. *R. tanezumi* and *R. nitidus* clustered together. Then, when *R. brunneusculus* and *N. confucianus* was added, the four host species clustered together; the rest of the communities were grouped separately (Figure 2).

Table 1. The 34 investigations and small mammal capture sites located in Ruili

Number	Abbreviation	Investigation	Number	Abbreviation	Investigation
1	YJ	Yinjing	18	MA	Mangai
2	MM	Mengmao	19	MS	Mangsha
3	MG	Manggun	20	NN	Nongnong
4	JL	Jiele	21	MZ	Mingzhai
5	GD	Guangdong	22	NC	Nongchang
6	ZZGS	Zhongzigongsi	23	JG	Jiegao
7	SNA	Shangnongan	24	HB	Hanban
8	HFGS	Haifenggongsi	25	JSL	Jianshelu
9	PAXQ	Pinganxiaoqu	26	SW	Shiwei
10	LJL	Lianjianlou	27	LCZ	Laochengzi
11	MaM	Manman	28	MeG	Mengga
12	NM	Nanmeng	29	JK	Jikong
13	TZC	Tuzaichang	30	MB	Mangbang
14	NP	Nongpian	31	LD	Liudui
15	SY	Suoyang	32	DX	Dengxiu
16	WD	Wudui	33	NX	Nongxiang
17	SD	Sidui	34	ML	Mangling

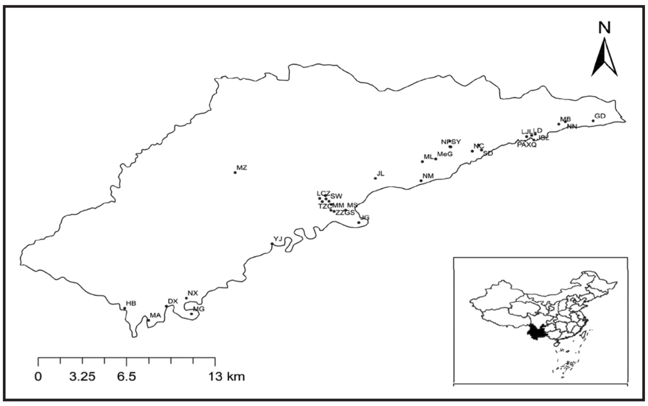

Figure 1. 34 survey sites for chigger mites in Ruili, Yunnan Province, China.

Table 2. Chigger mites on the surface of small animal hosts in Ruili, Yunnan Province, China

Subfamilies	Genera	Chigger species	Chigger individuals
Trombiculidae		6	4,941
	Leptotrombidium	1	118
	Microtrombicula	1	38
	Ascoschoengastia	1	85
	Schoengastiella	2	4,610
	Gahrliepia	1	90

Table 3. Major chigger mites infesting the hosts of small animals in Ruili, Yunnan Province, China

Name of chigger mites species	Number of hosts		Number of chiggers		Chigger mite infestations of hosts		
	Examined	Infested	Individuals	*Cr (%)*	*Pm (%)*	*MA*	*MI*
L. kunmingense	182	10	118	2.39	5.49	0.65	11.80
M. munda	182	2	38	0.77	1.10	0.21	19.00
A. indica	182	7	85	1.72	3.85	0.47	12.14
S. ligula	182	78	4,609	93.28	42.86	25.32	59.09
S. puntata	182	1	1	0.02	0.55	0.01	1.00
G. radiopunctata	182	7	90	1.82	3.85	0.49	12.86
Total	**182**	**86**	**4,941**	**100**	**47.25**	**27.15**	**57.45**

Table 4. Community structure of chigger mites on different small animals collected from Ruili, Yunnan Province, China

Name of hosts	Number of hosts			Community structure of chigger mites			
	Examined	Infested	*Pm (%)*	*S*	*H'*	*J'*	*D*
R. tanezumi	182	53	29.12	6	0.79	0.44	0.69
S. murinus	182	5	2.75	1	0	0	1
R. brunneusculus	182	5	2.75	2	0.04	0.06	0.99
B. indica	182	22	12.09	4	0.14	0.10	0.95
R. nitidus	182	0	0	0	0	0	0
N. confucianus	182	1	0.55	1	0	0	1
Total	**182**	**86**	**100**	**6**	**0.67**	**0.37**	**0.62**

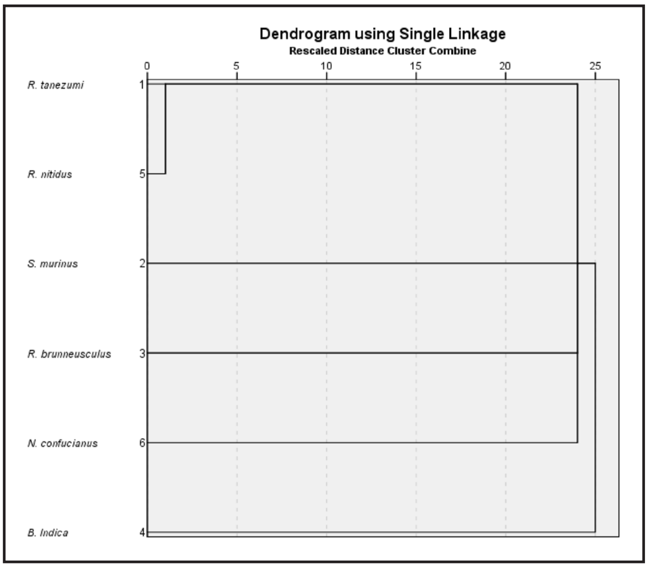

Figure 2. Stratified clustering tree diagram of seven major species of small animal in Ruili, Yunnani Province, China.

Analysis of the spatial distribution pattern of the chigger mite

A total of four spatial distribution indices were used to calculate the spatial distribution patterns of six species of chiggers on seven small animal hosts. Spatial distribution patterns were determined, and the results showed that the numbers of *L. kunmingense*, *A. indica*, *S. ligula* and *G. radiopunctata* were all greater than the threshold value for an aggregated distribution (m*/m>1), suggesting an aggregation distribution pattern, while the numbers for the remaining two species, *M. munda* and *S. puntata*, were less than the threshold value for aggregated distribution ($m^*/m<1$) and indicated uniform distributions (Table 5).

Analysis of interspecific relationships between dominant species of chiggers

Interspecific associations between any two chigger mite species were analyzed. The correlation coefficient (*V*) *was* -0.629 between *S. ligula* and *L. kunmingense* and was statistically significant ($P<0.05$). The *V* was 0.095 between *S. ligula* and *G. radiopunctata* and was statistically significant ($P < 0.05$). There was a negative correlation between *S. ligula* and *L. kunmingense* and positive correlation between *S. ligula* and *G. radiopunctata* (Table 6).

Chigger mite species compositions on different sexes

Among the 182 hosts captured, 181 had their definitive sex recorded, while the remaining host specimen had no sex recorded. Of the 181 hosts with sex records, 86 were male and 95 were female. In total, 3,140 chiggers were collected from 86 male hosts, and 1,801 chiggers were collected from 95 female hosts. The chigger mite *S* was five and six species for male and female hosts, respectively. The *Css* between male and female hosts was 90.91%. The *MA* and *MI* of chigger mites on females were 17.34 and 65.42, respectively, which were higher than those on males (9.95 and 47.39, respectively) ($P<0.05$). The *Pm* and *MA* of chigger mites on adult hosts were 32.41 and 18.59, which were both higher than those of chigger mites on infant and juvenile hosts, and the *MI* of chigger mites on adult hosts was higher than those of chigger mites on infant and juvenile hosts ($P<0.05$) (Table 7).

The infestation rate of chiggers on hosts showed some variations by latitude and longitude; the *Pm*, *MA* and *MI* of chiggers on hosts captured at < 24°N were 32.97, 23.18 and 70.30 times higher than those on hosts captured at 24°N-25°N ($P<0.05$). The *PM*, *MA* and *MI* of chigger mites on hosts captured at < 98°E were 40.11, 25.46 and 63.48 times higher than those on hosts captured at 98°E-99°E ($P<0.05$) (Table 8).

Chigger mite infestations on mammal hosts showed some variation with altitude; the highest *Pm*, *MA* and *MI* of chiggers on hosts captured at <800 m were 38.46, 25.05 and 65.13, respectively ($P<0.05$), and the lowest *Pm*, *MA* and *MI* of chiggers on hosts

Table 5. Means and variances (σ^2) for six species of chiggers *in vitro* in the host

Chigger species	*Mean*	σ^2	*C*	C_A	*I*	*m*/m*	Spatial distribution
L. kunmingense	59	2304	39.05	0.64	38.05	1.64	Aggregated distribution
M. munda	38	0	0	-0.03	-1.03	0.97	Uniform distribution
A. indica	42.5	1056.25	24.85	0.56	23.85	1.56	Aggregated distribution
S. ligula	921.8	1923523.76	2086.70	2.26	2085.70	3.26	Aggregated distribution
S. puntata	1	0	0	-1	-1	0	Uniform distribution
G. radiopunctata	30	1124.67	37.49	1.22	36.49	2.22	Aggregated distribution

Table 6. Analysis of the specific association of two dominant chigger mites in the mammal hosts *in vitro*

		L. kunmingense (Species x)			*G. radiopunctata*		
		+	–	Total	+	–	Total
S. ligula (Species y)	+	4 (a)	74 (b)	78 (a+b)	7 (a)	71 (b)	78 (a+b)
	_	6 (c)	2 (d)	8 (c+d)	0 (c)	8 (d)	8 (c+d)
	Total	10 (a+c)	76 (c+d)	86 (n)	7 (a+c)	79 (c+d)	86 (n)
V				-0.629			0.095
χ^2				28.008			42.487
P				*<0.05*			*<0.05*

* Chi-square (χ^2), Statistically significant ($P < 0.05$).

Table 7. Chigger mite of species compositions on different sexes and ages collected from Ruili, Yunnan Province, China

		Individual host	Collection of chigger mites		Overall infestations of chigger mites		
			Species	Individuals	*Pm (%)*	*MA*	*MI*
Sexes	Female	95	5	3,140	26.52	17.34	65.42
	Male	86	6	1,801	20.99	9.95	47.39
Ages	Infancy	15	2	33	2.20	0.18	8.25
	Juvenile	45	5	1,525	12.64	8.38	66.30
	Adult	122	6	3,383	32.41	18.59	57.34
	Total	182	6	4,941	47.25	27.15	57.45

Table 8. Chigger mite infestation on mammal hosts at different latitudes and longitudes of Yunnan Province, China

	Hosts		Number of chiggers		Infestations of hosts with chigger mites		
	Examined	Infested	Individuals	*Cr (%)*	*Pm (%)*	*MA*	*MI*
Latitude							
< 24°N	182	57	4,171	85.37	32.97	23.18	70.30
24°N-25°N	182	29	770	14.63	14.29	3.97	27.81
Longitude							
< 98°E	182	73	4,634	93.79	40.11	25.46	63.48
98°E-99°E	182	13	307	6.21	7.14	1.69	23.62
Total	**182**	**86**	**4,941**	**100**	**47.25**	**27.15**	**57.45**

Table 9. Infestation of chigger mites on mammal hosts at different altitudes at Ruili, Yunnan Province, China

Altitude (Meters)	Hosts		Number of chiggers		Infestations of hosts with chigger mites		
	Examined	Infested	Individuals	*Cr (%)*	*Pm (%)*	*MA*	*MI*
<800	182	70	4559	92.27	38.46	25.05	65.13
800-1000	182	12	325	6.58	6.59	1.79	27.08
>1000	182	4	57	1.15	2.20	0.31	14.25
Total	**182**	**86**	**4941**	**100**	**47.25**	**27.15**	**57.45**

captured at >1000 m were 2.20, 0.31 and 14.25, respectively ($P<0.05$) (Table 9).

DISCUSSION

The basic community structure of the chigger mite

In the current study, 4,941 chigger mites were collected from 182 hosts (rodents) at 34 survey sites in Ruili; the identified chigger mites belonged to five genera within one subfamily (Table 2). Among them, *S. ligula* was the dominant species ($P<0.001$). This is inconsistent with the results of the study by Zhu, who found that *L. deliense* was the dominant mite species in the western plateau subregion of Yunnan (Longchuan, Lianghe, Ruili, and Yingjiang) (Zhu *et al*., 2013). The different results may be due to different numbers of host samples; in the current study, six species of chigger mites were isolated from Rodentia hosts, which indicates that all of them are associated with Rodentia. *Schoengastiella ligula* was collected from different types of hosts, most of which were *R. tanezumi* and *B. indica*, indicating that the same species of chigger mite can infect different host animals. Moreover, the same host animal can harbor multiple chigger mite species. Low host specificity facilitates the transmission of pathogens, such as *Rickettsia tsutsugamushi*, between different host animals, hence increasing the risk of tsutsugamushi spread (Lv, 2021).

We found that the abundance and diversity of chigger mite species in Ruili, Yunnan Province, were low and the community structure was relatively simple. The dominant chigger mite species was relatively prominent; however, this may be related to the small number of rodent captures. In this study, hierarchical clustering analysis was performed and showed that the parasitic chigger communities on *R. tanezumi* and *R. nitidus* were clustered into one group. Moreover, the cluster relationships were consistent with the taxonomic status of the hosts. However, the clustering of chigger mite communities is not necessarily related to the taxonomic proximity of the hosts; rather, clustering seems to be related to host habitats, e.g., *B. indica* and *R. tanezumi* belong to the same family of rodents, but their chigger mite communities did not cluster together. This suggests that although there is some coevolutionary relationship between chigger mites and their hosts, the degree of coevolution is not high.

Determination of the spatial distribution patterns of chigger mites

In this study, four types of distribution indices were used to measure the spatial patterns of six species of chigger mites on small mammals. The results showed that the actual measured values of the four types of distribution indices for *L. kunmingense*, *A. indica*, *S. ligula* and *G. radiopunctata* were all higher than the critical value for an aggregated distribution (m^*/m). This indicates that all these species had an aggregation distribution pattern, which is a common pattern in many parasites. This distribution pattern is beneficial to survival, reproduction, and defense in parasites, and an aggregated distribution may be related to life habits and environmental factors (Huang *et al*., 2013; Liu *et al*., 2019; Lv, 2021). The lack of an aggregated distribution pattern in the other two chigger mite species, *M. munda* and *S. puntata*, may be due to the small number of captured hosts, the small numbers of these mites collected from certain hosts, or the calculated values being very close to the critical value (m^*/m), making it difficult to distinguish spatial patterns; this was a limitation in our methods.

Analysis of interspecific relationships between chigger mite species

In this study, the *V* was used to measure the interspecific relationship between any two chigger species, with a positive correlation indicating a tendency for the two species to coexist, a negative correlation indicating a tendency for the two species to not coexist, and no correlation indicating that the two species existed or were distributed independently (Liu *et al*., 2019; Chen *et al*., 2021; Yin *et al*., 2021). *Leptotrombidium kunmingense* and *S. ligula* were negatively correlated, suggesting that *L. kunmingense* and *S. ligula* tend to avoid inhabiting the same host, *G. radiopunctata* and *S. ligula* were positively correlated, indicating a tendency to coexist on the same host at the same time; the value of the *V* between the two chigger species ($P<0.05$) was statistically significant.

Host selection and distribution analysis of chigger mites

A comparison of male and female host infection statuses associated with the six species of chiggers showed that the prevalence of infection in their respective dominant hosts and all hosts was higher in adult females than in adult males, with statistically significant differences ($P<0.05$); this suggests a preference for host sex, which may be related to solitary reproduction, which is common in mites and some other arthropods (Huang *et al*., 2013). In terms of infection of hosts of different ages, adult host animals were more likely to be infected with chiggers than immature host animals, and the differences in the infection rate and *MA* for each mite species were statistically significant ($P<0.05$); this may be related to the fact that adult hosts are more active and have a longer window of exposure to the outside environment than immature hosts.

The results of the current study showed that the infection rate and the average abundance for each mite decreased with increasing latitude and longitude ($P<0.05$). The number of chigger mites on a host increased or decreased consistently with changing latitudes and longitudes for each chigger mite species, suggesting that latitude and longitude impacts the number of chigger mites on a host. Regarding the vertical distribution by altitude gradient, which in this survey ranged from 700-1800 m, 98.85% of the chigger mites were collected from the low-altitude area below 1000 m. The degree of infection by chigger mites decreased with increasing altitude, suggesting that chigger mites tended to be distributed in the low-altitude area. The warm and humid climate may be favorable for the growth and reproduction of chigger mites in the low-altitude area, which has a high temperature, abundant rainfall and relatively low atmospheric pressure; conversely, low temperature, low rainfall and high altitude have been reported as unfavorable for the growth and reproduction of chigger mites (Peng *et al*., 2022).

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Conflict of interest

The authors declare that there are no relevant conflicts of interest in this study.

REFERENCES

Chen, Y.L., Guo, X.G., Ren, T.G., Zhang, L., Fan, R., Zhao, C.F., Zhang, Z.W., Mao, K.Y., Huang, X.B. & Qian, T.J. (2021). A report of chigger mites on the Stripedfield Mouse, *Apodemus agrarius*, in Southwest China. *Korean Journal of Parasitology* **59**: 625-634. https://doi.org/10.3347/kjp.2021.59.6.625

Chen, Y.L., Guo, X.G., Ren, T.G., Zhang, L., Fan, R., Zhao, C.F., Zhang, Z.W., Mao, K.Y., Huang, X.B. & Qian, T.J. (2022). Infestation and distribution of chigger mites on Chevrieri's field mouse (*Apodemus chevrieri*) in Southwest China. *International Journal for Parasitology. Parasites and Wildlife* **17**: 74-82. https://doi.org/10.1016/j.ijppaw.2021.12.003

Ding, F., Guo, X.G., Song, W.Y., Fan, R., Zhao, C.F., Mao, K.Y., Zhang, Z.W., Peng, P.Y., Lin, H., Dong, W.G. *et al*. (2021a). Infestation and distribution of chigger mites on Brown rat (*Rattus norvegicus*) in Yunnan Province, Southwest China. *Tropical Biomedicine* **38**: 111-121. https://doi.org/10.47665/tb.38.1.020

Ding, F., Jiang, W.L., Guo, X.G., Fan, R., Mao, K.Y., Zhao, C.F., Zhang, Z.W., Qian, T.J. & Yang, Z.H. (2020). A preliminary report on *Walchia microplate* in Yunnan Province. *Sichuan Journal of Zoology* **39**: 555-562.

Ding, F., Jiang, W.L., Guo, X.G., Fan, R., Zhao, C.F., Zhang, Z.W., Mao, K.Y. & Xiang, R. (2021b). Infestation and related ecology of chigger mites on the Asian House Rat (*Rattus tanezumi*) in Yunnan Province, Southwest China. *Korean Journal of Parasitology* **59**: 377-392. https://doi.org/10.3347/kjp.2021.59.4.377

Guo, X.G., Qian, T.J., Meng, X.Y., Dong, W.G., Shi, W.X. & Wu, D. (2006). Preliminary analysis of chigger communities associated with house rats (*Rattus flavipectus*) from six counties in Yunnan, China. *Systematic and Applied Acarology* **11**: 13-21. https://doi.org/10.11158/saa.11.1.2

Guo, X.G., Speakman, J.R., Dong, W.G., Men, X.Y., Qian, T.J., Wu, D., Qin, F. & Song, W.Y. (2013). Ectoparasitic insects and mites on Yunnan red-backed voles (*Eothenomys miletus*) from a localized area in southwest China. *Parasitology Research* **112**: 3543-3549. https://doi.org/10.1007/s00436-013-3537-6

Huang, L.Q., Guo, X.G., Speakman, J.R. & Dong, W.G. (2013). Analysis of gamasid mites (Acari: Mesostigmata) associated with the Asian house rat, *Rattus tanezumi* (Rodentia: Muridae) in Yunnan Province, southwest China. *Parasitology Research* **112**: 1967-1972. https://doi.org/10.1007/s00436-013-3354-y

Lee, I.Y., Kim, H.C., Lee, Y.S., Seo, J.H., Lim, J.W., Yong, T.S., Klein, T.A. & Lee, W.J. (2009). Geographical distribution and relative abundance of vectors of scrub typhus in the republic of Korea. *Korean Journal of Parasitology* **47**: 381-386. https://doi.org/10.3347/kjp.2009.47.4.381

Lee, I.Y., Lim, J.W., Seo, J.H., Kim, H.C., Lee, K.J., Yong, T.S., Lee, W.J., Yu, J.R. & Sim, S. (2021). Geographical distribution and epidemiologicfactors of chigger mites on apodemus agrarius during autumn in Korea. *Korean Journal of Parasitology* **59:** 473-479. https://doi.org/10.3347/kjp.2021.59.5.473

Li, W., Niu, Y.L., Zhao, Z., Ren, H.Y., Li, G.C., Liu, X.B., Gao, Y., Wang, J., Lu, L. & Liu, Q.Y. (2021). Meteorological factors and related lag effects on scrub typhus in southwestern Yunnan. *Chinese Journal of Epidemiology* **42**: 1235-1239.

Liu, X.H. & Ren, T.G. (2022). The list of new species and new record chigger mites of China. *China Tropical Medicine* **22**: 512-516.

Liu, Z., Guo, X.G., Fan, R., Zhao, C.F., Mao, K.Y., Zhang, Z.W. & Zhao, Y. (2019). Ecological analysis of gamasid mites on the body surface of Norway rats *(Rattus norvegicus*) in Yunnan Province, Southwest China. *Biologia* **75**: 1325-1336. https://doi.org/10.2478/s11756-019-00383-z

Lv, Y., Guo, X.G., Jin, D.C., Song, W.Y, Peng, P.Y., Lin, H., Fan, R., Zhao, C.F., Zhang, Z.W., Mao, K.Y. *et al*. (2021). Infestation and seasonal fluctuation of chigger mites on the Southeast Asian house rat (*Rattus brunneusculus*) in southern Yunnan Province, China. *International Journal for Parasitology: Parasites and Wildlife* **14**: 141-149. https://doi.org/10.1016/j.ijppaw.2021.02.005

Lv, Y. (2021). Distribution patterns of *Leptotrombidium deliense* and other important chigger mites in southwest China. Guizhou University, pp. 1-143.

Lv, Y., Guo, X.G. & Jin, D.C. (2018). Research Progress on *Leptotrombidium deliense*. *Korean Journal of Parasitology* **56**: 313-324. https://doi.org/10.3347/kjp.2018.56.4.313

Peng, P.Y., Guo, X.G., Ren, T.G., Dong, W.G. & Song, W.Y. (2016a). An updated distribution and hosts: trombiculid mites (Acari: Trombidiformes) associated with small mammals in Yunnan Province, southwest China. *Parasitology Research* **115**: 1923-1938. https://doi.org/10.1007/s00436-016-4934-4

Peng, P.Y., Guo, X.G., Ren, T.G., Song, W.Y., Dong, W.G. & Fan, R. (2016b). Species diversity of ectoparasitic chigger mites (Acari: Prostigmata) on small mammals in Yunnan Province, China. *Parasitology Research* **115**: 3605-3618. https://doi.org/10.1007/s00436-016-5127-x

Peng, P. Y. (2019). Species diversity and faunal taxonomic studies of gamasid mites on small mammals in Yunnan (Acari: Mesostigmata: Gamasina). Guizhou University, pp. 1-165.

Peng, P.Y., Guo, X.G., Song, W.Y., Hou, P., Zou, Y.J., Fan, R. & He, X.S. (2015). Analysis of ectoparasites (chigger mites, gamasid mites, fleas and sucking lice) of the Yunnan red-backed vole (*Eothenomys miletus*) sampled throughout its range in southwest China. *Medical and Veterinary Entomology* **29**: 403-415. https://doi.org/10.1111/mve.12134

Peng, P.Y., Xu, L., Wang, G.X., He, W.Y., Yan, T.L. & Guo, X.G. (2022). Epidemiological characteristics and spatiotemporal patterns of scrub typhus in Yunnan Province from 2006 to 2017. *Scientific Reports* **12**: 2985. https://doi.org/10.1038/s41598-022-07082-x

Shen, S., Xu, G., Li, D., Clements, D.R., Zhang, F., Jin, G., Wu, J., Wei, P., Lin, S. & Xue, D. (2017). Agrobiodiversity and in situ conservation in ethnic minority communities of Xishuangbanna in Yunnan Province, Southwest China. *Journal of Ethnobiology and Ethnomedicine* **13**: 28. https://doi.org/10.1186/s13002-017-0158-7

Xiang, R. & Guo, X.G. (2021). Research advances of *Leptotrombidium scutellare* in China. *Korean Journal of Prasitology* **59**: 1-8. https://doi.org/10.3347/kjp.2021.59.1.1

Xin, H., Sun, J., Yu, J., Huang, J., Chen, Q., Wang, L., Lai, S., Clements, A.C.A., Hu, W. & Li, Z. (2020). Spatiotemporal and demographic characteristics of scrub typhus in Southwest China, 2006-2017: An analysis of population-based surveillance data. *Transboundary and Emerging Diseases* **67**: 1585-1594. https://doi.org/10.1111/tbed.13492

Xu, G., Walker, D.H., Jupiter, D., Melby, P.C. & Arcari, C.M. (2017). A review of the global epidemiology of scrub typhus. *PLoS Neglected Tropical Diseases* **11**: e0006062. https://doi.org/10.1371/journal.pntd.0006062

Yao, H., Wang, Y., Mi, X., Sun, Y., Liu, K., Li, X., Ren, X., Geng, M., Yang, Y., Wang, L. *et al*. (2019). The scrub typhus in mainland China: spatiotemporal expansion and risk prediction underpinned by complex factors. *Emerging Microbes & Infections* **8**: 909-919. https://doi.org/10.1080/22221751.2019.1631719

Yin, P.W., Guo, X.G., Jin, D.C., Fan, R., Zhao, C.F., Zhang, Z.W., Huang, X.B. & Mao, K.Y. (2021). Distribution and Host Selection of Tropical Rat Mite, *Ornithonyssus bacoti*, in Yunnan Province of Southwest China. *Animals* **11**: 110. https://doi.org/10.3390/ani11010110

Yue, Y., Ren, D., Liu, X., Wang, Y., Liu, Q. & Li, G. (2019). Spatio-temporal patterns of scrub typhus in mainland China, 2006-2017. *PLoS Neglected Tropical Diseases* **13**: e0007916. https://doi.org/10.1371/journal.pntd.0007916

Zhan, Y.Z., Guo, X.G., Speakman, J.R., Zuo, X.H., Wu, D., Wang, Q.H. & Yang, Z.H. (2013). Abundances and host relationships of chigger mites in Yunnan Province, China. *Medical and Veterinary Entomology* **27**: 194-202. https://doi.org/10.1111/j.1365-2915.2012.01053.x

Zhang, S.Y., Guo, X.G., Gong, Z.D., Wu, D. & Wang, Z.K. (2009). Investigation on similarity and coevolution of flea communities on small mammals of 19 counties in Yunnan China. *Acta Parasitology et Medica Entomologica Sinica* **16**: 38-42.

Zhu, Q.R., Guo, X.G., Huang, H., Dong, W.G. & Zhao, N. (2013). Geographical distribution of chigger mites on *Rattus flavipectusin* Yunnan Province. *Chinese Journal of Parasitology and Parasitic Diseases* **31**: 395-399+405.