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Infestation and seasonal fluctuation of chigger mites on the Southeast Asian house rat (*Rattus brunneusculus*) in southern Yunnan Province, China

Yan Lv [a,b], Xianguo Guo [a,b,*], Daochao Jin [b], Wenyu Song [a], Peiying Peng [a], Hao Lin [a], Rong Fan [a], Chengfu Zhao [a], Zhiwei Zhang [a], Keyu Mao [a], Tijun Qian [a], Wenge Dong [a], Zhihua Yang [a]

[a] *Vector Laboratory, Institute of Pathogens and Vectors, Yunnan Provincial Key Laboratory for Zoonosis Control and Prevention, Dali University, Dali, Yunnan Province, 671000, China*

[b] *Institute of Entomology, Guizhou University, the Provincial Key Laboratory for Agricultural Pest Management in Mountainous Region, Guiyang, 550025, China*

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ABSTRACT

Chigger mites are the common ectoparasites of rodents and the exclusive vector of scrub typhus. The Southeast Asian house rat (*Rattus brunneusculus*) is an important reservoir host and infectious source of some zoonoses including scrub typhus. From April 2016 to March 2017, a 12-month consecutive investigation was made at Jingha village in southern Yunnan of China, which is an important focus of scrub typhus. The infestation and seasonal fluctuation of chigger mites on *R. brunneusculus* were studied based on the investigation. From 2,053 captured *R. brunneusculus*, a total of 99,221 chiggers were collected and identified as comprising 102 species with very high species diversity. The richness (*S*), diversity index (*H′*), evenness (*E*) and dominance index (*D*) of the chigger community on the rat varied in different months. Of the 102 chigger species, five main species accounted for 84.81% of the total chiggers (84,147/99,221). The five main chiggers were *Walchia* (*W.*) *micropelta* (32.65%), *Ascoschoengastia indica* (24.68%), *Leptotrombidium* (*L.*) *deliense* (19.02%), *W.* (*W.*) *turmalis* (4.63%) and *L.* (*L.*) *scutellare* (3.83%). Of the five chigger species, *L.* (*L.*) *deliense* and *L.* (*L.*) *scutellare* are the most important vectors of scrub typhus in China. The five chigger species showed different patterns of seasonal fluctuation. The seasonal fluctuation of *L.* (*L.*) *deliense* belonged to summer-autumn type with the highest peak in July, but *L.*(*L.*) *scutellare* mainly appeared in winter and spring with the peak from January to February. The temperature and rainfall were two key factors which influenced the seasonal fluctuation of chigger mites.

1. Introduction

Chigger mites are a large group of arthropods with a unique mode of parasitism among medically-relevant arthropods and their larvae (often known as chiggers) are the exclusive ectoparasitic stage in their complex lifec cycle (Zhang et al., 2011; Walter et al., 2009; Santibáñez et al., 2015; Chaisiri et al., 2019). Most stages of chigger mites are edaphic creatures and some of them (deutonymphs and adults) are predators of some other arthropods (especially arthropod eggs) in the soil (Chaisiri et al., 2019; Shatrov and Kudryashova, 2006; Li et al., 1997). Chiggers are common ectoparasites on vertebrates (occasionally some invertebrates), and rodents and some other small mammals are their common hosts (Elliott et al., 2019; Daniel and Stekolnikov, 2009; Lv et al., 2019). As the exclusive vector of scrub typhus (tsutsugamushi disease) caused by the agent *Orientia tsutsugamushi* (Ot), some chigger species can transmit the disease among different hosts through their biting activity (Li et al., 1997; Santibáñez et al., 2015; Lv et al., 2019; Peng et al., 2018). In addition, some chiggers are suspected to be associated with the transmission of hemorrhagic fever with renal syndrome (HFRS) caused by different types of hantaviruses under Bunyaviridae (Wu et al., 1996; Li et al., 1997; Lv et al., 2019; Peng et al., 2018). Scrub typhus is a zoonotic disease potentially threatening human health and it is widely prevalent in Asian Pacific regions where more than one billion people are at risk of being infected and around one million new cases are reported annually (Bonell et al., 2017). In recent years, the prevalence of the disease in many places has shown a rapid increase, and the epidemic foci have been continuously expanding (Chaisiri et al., 2019; Elliott et al., 2019; Tilak and Kunte, 2019). Scrub typhus was previously believed to be only associated with Asian Pacific regions (Bonell et al., 2017), but it has probably spread to some other places of the world in

* Corresponding author. Vector Laboratory, Institute of Pathogens and Vectors, Yunnan Provincial Key Laboratory for Zoonosis Control and Prevention, Dali University, Dali, Yunnan Province, 671000, China.

E-mail address: xianguoguo@yahoo.com (X. Guo).

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recent years. For example, endemic scrub typhus has been reported from United Arab Emirates (Izzard et al., 2010) and Chile (Weitzel et al., 2016), and local transmission is suspected in Kenya (Masakhwe et al., 2018).

Scrub typhus is also widespread in China and its prevalence has been increasing with gradually expanded epidemic foci (Elliott et al., 2019; Wu et al., 2015). The disease is mainly prevalent in the vast areas south of the Yangtze River (e.g. Guangdong, Fujian, Hainan, Taiwan and Yunnan) (Su et al., 2012; Wu et al., 2013). Yunnan Province, especially southern Yunnan, is one of the main foci of scrub typhus in China (Yuan et al., 2018). There were 1208 cases of scrub typhus reported in Xishuangbanna prefecture in southern Yunnan between 2006 and 2017 (Yuan et al., 2018). The investigated site (Jingha village) of the present study is located in Xishuangbanna, an epidemic foci of scrub typhus.

The Southeast Asian house rat, *Rattus brunneusculus*, was named by Hodgson in 1845. Although some scholars considered *R. brunneusculus* a synonym of the Asian house rat, *R. tanezumi* Temminck, 1844 (Alfred, 2005; Ellerman, 1961; Wilson and Reeder, 2005), more scientists believe that *R. brunneusculus* is an independent rat species, which is obviously different from *R. tanezumi* in morphology (Dhananjoy et al., 2014a,b; Gao et al., 2017; Wang, 2003). The Southeast Asian house rat is not only an important agricultural and forestry pest, but also an important reservoir host and infection source of some zoonoses (plague, HFRS, and scrub typhus, etc.) (Chauhan and Saxena, 1987; Dong et al., 2009). Based on the 12 months' investigation at Jingha village of southern Yunnan between April 2016 and March 2017, the present study analyzed the infestation of *R. brunneusculus* with chigger mites (especially five main species) and the seasonal fluctuations of the mites. The five main chigger species are *Walchia* (*Walchia*) *micropelta* (Traubet Evans, 1957), *W.* (*W.*) *turmalis* (Gater, 1932), *Ascoschoengastia indica* (Hirst, 1915), *Leptotrombidium* (*Leptotrombidium*) *deliense* (Walch, 1922) and *L.* (*L.*) *scutellare* (Nagayo et al., 1921). Previously *Walchia* (*W.*) *micropelta* and *W.* (*W.*) *turmalis* were once named *Gahrliepia micropelta* and *G. turmalis* (Traub and Evans, 1957; Gater, 1932), and *A. indica* was once named *Schongastia indica* (Hirst, 1915). *Leptotrombidium* (*L.*) *deliense* and *L.* (*L.*) *scutellare* were originally named *Trombicula deliensis* and *T. scutellare* (Walch, 1922; Nagayo et al., 1921).

2. Materials and methods

2.1. Field investigation

From April 2016 to March 2017, a 12-month consecutive field investigation was made at Jingha village, Jinghong county, Xishuangbanna prefecture in the south of Yunnan province. Each month's investigation lasted 15–20 days. Located at 21°50′ north latitude and 100°52′ east longitude with an altitude of 500–700 m, Jingha village is a typical valley and flatland area near the coast of the Lancang River, a river from the northwest to the south in Yunnan province (Sun et al., 2000; Yu et al., 2008). The village is a rubber planting area with lots of rubber woodlands dotted with some banana fields, farmlands, bush areas and broad-leaved forests. The online meteorological data was provided by the local weather forecasting department. The data of 2016 was from the websites: https://tianqi.911cha.com/jinghong/2016.html, and the data of 2017 from the website: https://tianqi.911cha.com/jinghong/2017.html.

2.2. Collection and identification of chigger mites and their hosts

The animal hosts (rodents and some other small mammals) of chiggers were mainly captured with mousetraps (18 × 12 × 9 cm, Guixi Mousetrap Apparatus Factory, Guixi, Jiangxi, China). The mousetraps were set in the former evening and checked in the next morning. Every collected animal host was separately placed in a white cloth bag and brought to the laboratory where the host was anesthetized with ether. Over a large white tray, chiggers were collected from each host with a special bistoury or curette (Lv et al., 2019; Peng et al., 2016). After the collection of chiggers, every host was identified into species according to its body size, body shape, body color and some measurements such as the body weight, body length and the lengths of ears, tail and hind feet (Kia et al., 2009; Wang, 2003; Wilson and Reeder, 2005). The capture of small mammals was officially approved by the wildlife administration of local governments. The use of animals for the research (including rodent euthanasia) was also formally approved by Ethics Committee of Dali University, which followed the international standards of animal euthanasia, 2013 AVMA guidelines (Cima, 2013).

In the laboratory, the preserved chiggers in 70% of ethanol were isolated from some other "non-mite" impurities, the scurf and debris from the rats' skin, under a stereo microscope, and then made into slide-mounted specimens with Hoyer's medium. With the help of some relevant taxonomic literatures including taxonomic monographs and identification keys (Traub and Morrow, 1955; Traub and Evans, 1957; Nadchatram and Traub, 1971; Vercammen-Grandjean and Langston, 1976; Nadchatram et al., 1980; Goff et al., 1982; Ree, 1990; Li et al., 1997; Fernandes and Kulkarni, 2003; Stekolnikov, 2013; Stekolnikov and González-Acuña, 2015; Chaisiri et al., 2016), the slide-mounted chiggers were identified to species under microscopes after dehydration and transparent process. The specimens of chiggers and representative rats were deposited in Institute of Pathogens and Vectors, Dali University, China.

2.3. Infestation statistics and analysis

On the basis of counting the total number of chigger species and the individuals of each chigger species, the constituent ratio (*Cr*), prevalence (P_M), mean abundance (*MA*) and mean intensity (*MI*) were used to calculate the infestations of the Southeast Asian house rat with chiggers. The *Cr* (%) is the percentage of each chigger species, P_M (%) the percentage of infested hosts (*R. brunneusculus*), *MA* the chiggers per examined rat host (mites/rat) and *MI* the chiggers per infested host (mites/rat) (Bush et al., 1997; Peng et al., 2018). Pearson's linear correlation was used to analyze the relationship between infestations of *R. brunneusculus* with chiggers and climatic factors (temperature, humidity and rainfall) in 12 months (Lv et al., 2019).

2.4. Community structure analysis

The richness index (richness, *S*), Shannon-Wiener's diversity index (*H*′), Pielou's evenness (*E*) and Simpson's dominance index (*D*) were used to describe the chigger community structure (Zhan et al., 2013).

$$S = \sum S_i; \quad H' = -\sum_{i=1}^{S}\left(\frac{N_i}{N}\right)\ln\left(\frac{N_i}{N}\right); \quad E = \frac{H'}{\ln S}; \quad D = \sum_{i=1}^{S}\left(\frac{N_i}{N}\right)^2$$

In the above formulas, S_i stands for chigger species *i* in the chigger community, N_i the number of chigger species *i* and *N* the total number of all chiggers.

3. Results

3.1. Infestation of the Southeast Asian house rat (Rattus brunneusculus) with chiggers

From 2,053 Southeast Asian house rats, a total of 99,221 chiggers were collected and they were identified as comprising 102 species with a high overall prevalence (P_M = 89.87%), mean abundance (*MA* = 48.33 mites/rat) and mean intensity (*MI* = 53.78 mites/rat). The majority of chiggers were from May to October and the monthly fluctuation of all infestation parameters showed a slight peak in July (*Cr* = 12.32%; P_M = 94.74%; *MA* = 80.42 mites/rat; *MI* = 84.89 mites/rat) (Table 1, Fig. 1). The chigger community on *R. brunneusculus* also showed some monthly variations in species richness indices (*S*: 24–44), Shannon-Wiener's

Table 1
Seasonal fluctuation of overall infestations of the Southeast Asian house rat (*Rattus brunneusculus*) with chiggers at Jingha village in southern Yunnan of China (2016–2017).

Months	Examined small mammal hosts		Collected chiggers		Overall infestations of *R. brunneusculus* with chiggers		
	No. of hosts	*Cr* (%)	No. of mites	*Cr* (%)	P_M (%)	*MA* (mites/rat)	*MI* (mites/rat)
1	182	8.87	6682	6.73	86.81	36.71	42.29
2	167	8.13	7131	7.19	87.43	42.70	48.84
3	182	8.87	8097	8.16	89.56	44.49	49.67
4	168	8.18	7119	7.17	86.31	42.38	49.10
5	184	8.96	11221	11.31	86.96	60.98	70.13
6	150	7.31	7725	7.79	94.67	51.50	54.40
7	152	7.40	12224	12.32	94.74	80.42	84.89
8	151	7.36	9003	9.07	91.39	59.62	65.24
9	141	6.87	7616	7.68	92.91	54.01	58.14
10	190	9.25	9400	9.47	91.05	49.47	54.34
11	197	9.60	6996	7.05	86.29	35.51	41.15
12	189	9.21	6007	6.05	92.59	31.78	34.33
Total	2053	100.00	99221	100.00	89.87	48.33	53.78

Annotation: The field investigation at Jingha village was made between April 2016 and March 2017, which forms a consecutive process from January to December.

diversity indices (*H*': 1.398–2.210) and Pielou's evenness indices (*E*: 0.434–0.599), but no obvious peaks were found (Table 2). Of 102 chigger species, five species were the most abundant and they accounted for 84.81% (84,147/99,221) of the total chiggers. The five main chigger species were *W.* (*W.*) *micropelta* (32.65%), *A. indica* (24.68%), *L.* (*L.*) *deliense* (19.02%), *W.* (*W.*) *turmalis* (4.63%) and *L.* (*L.*) *scutellare* (3.83%), and they had a high prevalence (P_M: 23.77%–75.65%), mean abundance (*MA*: 1.85–15.78) and mean abundance (*MI*: 4.69–20.86) (Table 3).

3.2. Seasonal fluctuation of five main chigger species on R. brunneusculus

On the basis of calculating the constituent ratio (*Cr*), prevalence (P_M), mean abundance (*MA*) and mean intensity (*MI*), the seasonal fluctuations of infestations of *R. brunneusculus* with five main chigger species were summarized in Table 4.

Walchia (*W.*) *micropelta* could be found throughout the year with an irregular seasonal fluctuation. Its *Cr*, *MA* and *MI* were relatively high from March to July with a slight peak in May (*Cr* = 15.37%; *MA* = 27.06 mites/rat; *MI* = 36.61 mites/rat), and its P_M was highest in July (P_M = 84.21%). The lowest chigger infestations occurred in December (*Cr* = 3.88%; P_M = 65.61%; *MA* = 6.65 mites/rat; *MI* = 10.14 mites/rat) (Table 4, Fig. 2).

Ascoschoengastia indica appeared throughout the year and it had an obvious seasonal fluctuation with two peaks. The first peak of *Cr*, *MA*

Table 2
Seasonal fluctuation of community parameters of chiggers on the Southeast Asian house rat (*R. brunneusculus*) at Jingha village in southern Yunnan of China (2016–2017).

Years and months		Community structure of chiggers			
Years	Months	*S*	*H*′	*E*	*D*
2017	1	31	1.898	0.553	0.229
	2	42	1.991	0.533	0.257
	3	38	1.676	0.461	0.329
2016	4	44	1.762	0.466	0.267
	5	24	1.545	0.486	0.278
	6	25	1.398	0.434	0.318
	7	34	1.541	0.437	0.278
	8	25	1.554	0.483	0.345
	9	31	1.792	0.522	0.230
	10	30	1.621	0.477	0.262
	11	35	1.958	0.551	0.215
	12	40	2.210	0.599	0.149
Total		102	2.080	0.450	0.208

Annotation: Same as in Table 2.

Table 3
Infestations of the Southeast Asian house rat (*R. brunneusculus*) with five main chigger species at Jingha village in southern Yunnan of China (2016–2017).

Five main chigger species	Constituent ratios of chiggers		Infestations of *R. brunneusculus* with chiggers		
	Individuals	*Cr* (%)	P_M (%)	*MA* (mites/rat)	*MI* (mites/rat)
W. micropelta	32395	32.65	75.65	15.78	20.86
A. indica	24490	24.68	68.73	11.93	17.36
L. deliense	18867	19.02	68.73	9.19	13.37
W. turmalis	4597	4.63	47.78	2.24	4.69
L. scutellare	3798	3.83	23.77	1.85	7.78
Total	84147	84.81			

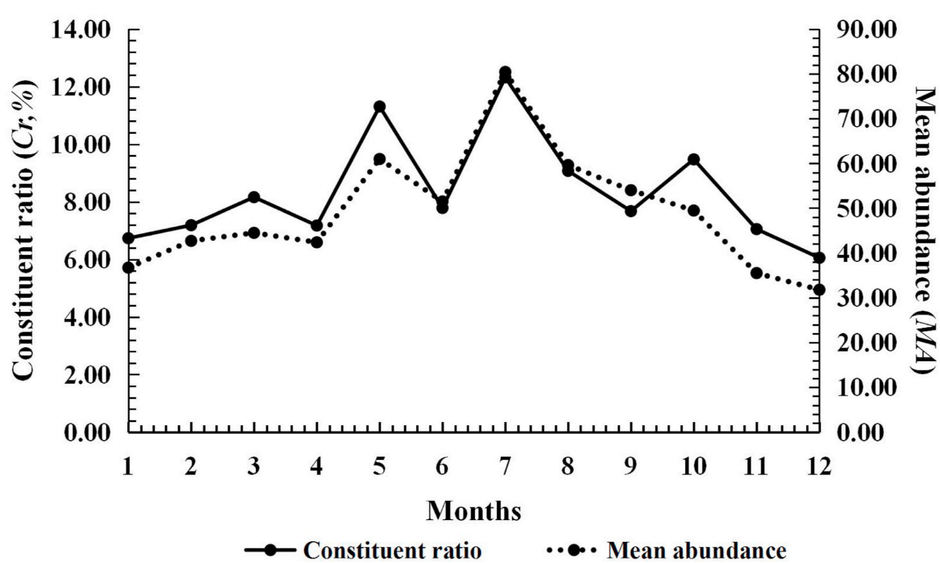

Fig. 1. Seasonal fluctuation of overall infestations of the Southeast Asian house rat (*R. brunneusculus*) with chiggers at Jingha village in southern Yunnan of China (April 2016–March 2017).

Table 4
Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. brunneusculus*) with five main chigger species at Jingha village in southern Yunnan of China (2016–2017).

Years		2017			2016									Total
Months		1	2	3	4	5	6	7	8	9	10	11	12	
Examined hosts		182	167	182	168	184	150	152	151	141	190	197	189	2053
W. (*W.*) *micropelta*	mites	2332	3337	4375	3101	4979	2314	3777	1408	1775	2239	1501	1257	32395
	Cr (%)	7.20	10.30	13.51	9.57	15.37	7.14	11.66	4.35	5.48	6.91	4.63	3.88	100.00
	P_M (%)	77.47	77.84	78.02	79.76	73.91	78.00	84.21	75.50	81.56	74.74	65.99	65.61	75.65
	MA	12.81	19.98	24.04	18.46	27.06	15.43	24.85	9.32	12.59	11.78	7.62	6.65	15.78
	MI	16.54	25.67	30.81	23.14	36.61	19.78	29.51	12.35	15.43	15.77	11.55	10.14	20.86
A. indica	mites	1860	486	1069	1698	2494	1268	2457	4956	2861	3324	1639	378	24490
	Cr (%)	7.59	1.98	4.37	6.93	10.18	5.18	10.03	20.24	11.68	13.57	6.69	1.54	100.00
	P_M (%)	67.58	49.10	66.48	60.71	67.93	68.00	78.95	86.75	89.36	76.84	66.50	53.97	68.73
	MA	10.22	2.91	5.87	10.11	13.55	8.45	16.16	32.82	20.29	17.49	8.32	2.00	11.93
	MI	15.12	5.93	8.83	16.65	19.95	12.43	20.48	37.83	22.71	22.77	12.51	3.71	17.36
L. (*L.*) *deliense*	mites	183	11	303	719	1186	3445	4553	927	1136	2605	2315	1484	18867
	Cr (%)	0.97	0.06	1.61	3.81	6.29	18.26	24.13	4.91	6.02	13.81	12.27	7.87	100.00
	P_M (%)	43.96	6.59	56.59	61.90	67.39	90.00	88.82	80.13	85.82	85.79	80.71	82.01	68.73
	MA	1.01	0.07	1.66	4.28	6.45	22.97	29.95	6.14	8.06	13.71	11.75	7.85	9.19
	MI	2.29	1.00	2.94	6.91	9.56	25.52	33.73	7.66	9.39	15.98	14.56	9.57	13.37
W. (*W.*) *turmalis*	mites	118	165	293	626	1443	367	487	231	435	71	225	136	4597
	Cr (%)	2.57	3.59	6.37	13.62	31.39	7.98	10.59	5.03	9.46	1.54	4.89	2.96	100.00
	P_M (%)	33.52	41.32	53.85	57.74	63.04	51.33	65.13	50.33	73.05	23.16	39.59	33.33	47.78
	MA	0.65	0.99	1.61	3.73	7.84	2.45	3.20	1.53	3.09	0.37	1.14	0.72	2.24
	MI	1.93	2.39	2.99	6.45	12.44	4.77	4.92	3.04	4.22	1.61	2.88	2.16	4.69
L. (*L.*) *scutellare*	mites	1060	1051	956	10	1	0	1	0	30	0	0	689	3798
	Cr (%)	27.91	27.67	25.17	0.26	0.03	0.00	0.03	0.00	0.79	0.00	0.00	18.14	100.00
	P_M (%)	66.48	61.68	67.58	2.38	0.54	0.00	0.66	0.00	16.31	0.00	0.00	59.26	23.77
	MA	5.82	6.29	5.25	0.06	0.01	0.00	0.01	0.00	0.21	0.00	0.00	3.65	1.85
	MI	8.76	10.20	7.77	2.50	1.00	–	1.00	–	1.30	–	–	6.15	7.78

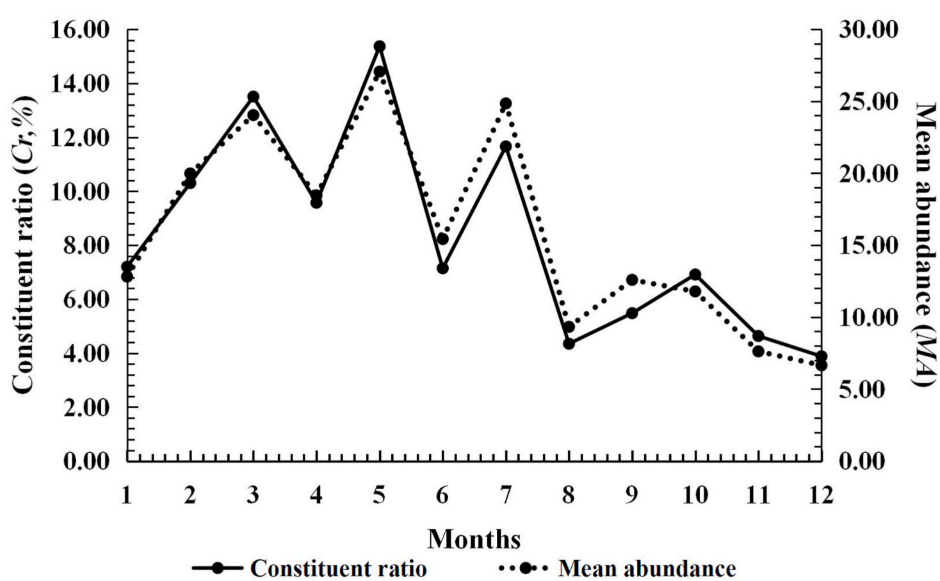

Fig. 2. Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. brunneusculus*) with *Walchia* (*W.*) *micropelta* at Jingha, southern Yunnan of China (April 2016–March 2017).

and *MI* in May ($Cr = 10.18\%$; $MA = 13.55$ mites/rat; $MI = 19.95$ mites/rat) was much lower than the second peak (highest peak of the whole year) in August ($Cr = 20.24\%$; $MA = 32.82$ mites/rat; $MI = 37.83$ mites/rat). The highest P_M, however, was in September ($P_M = 89.36\%$). The *Cr*, *MA* and *MI* decreased from September and reached the lowest level in December ($Cr = 1.54\%$; $MA = 2.00$ mites/rat; $MI = 3.71$ mites/rat), but the P_M was lowest in February ($P_M = 49.10\%$) (Table 4, Fig. 3).

Leptotrombidium (*L.*) *deliense* also appeared throughout the year and it had an obvious seasonal fluctuation with two peaks. The infestation parameters remained at a low level from January to April, and then rapidly rose from May on. The *Cr, MA* and *MI* reached the highest peak of the whole year (the first peak) in July ($Cr = 24.13\%$; $MA = 29.95$ mites/rat; $MI = 33.73$ mites/rat). The highest P_M, however, was in June ($P_M = 90.00\%$). The second peak of *Cr*, *MA* and *MI* appeared in October ($Cr = 13.81\%$; $MA = 13.71$ mites/rat; $MI = 15.98$ mites/rat), but it was much lower than the first peak in July (Table 4, Fig. 4).

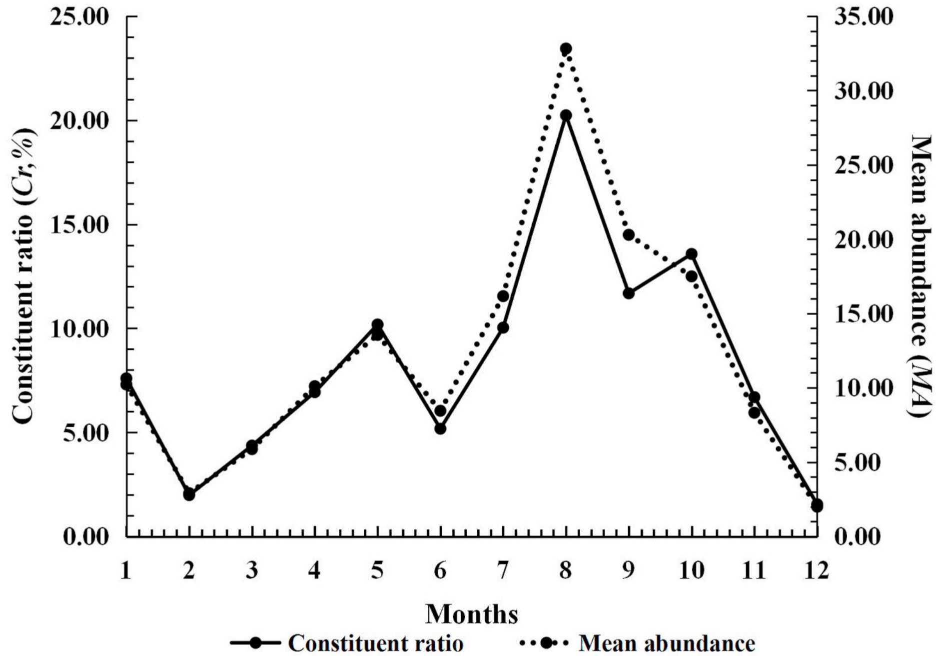

Fig. 3. Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. brunneusculus*) with *Ascoschoengastia indica* at Jingha, southern Yunnan of China (April 2016–March 2017).

Walchia (*W.*) *turmalis* appeared throughout the year. Its *Cr*, *MA* and *MI* gradually increased from January to April, and then reached the highest peak of the whole year in May ($Cr = 31.39\%$; $MA = 7.84$ mites/rat; $MI = 12.44$ mites/rat). The highest P_M, however, was in September ($P_M = 73.05\%$). From June to December, most infestation parameters remained at a very low level, and they were the lowest in October ($Cr = 1.54\%$; $P_M = 23.16\%$; $MA = 0.37$ mites/rat; $MI = 1.61$ mites/rat) (Table 4, Fig. 5).

All the infestation parameters of *L.* (*L.*) *scutellare* were very low from April to November, and they remained at the lowest level of the whole year. From December on, these parameters quickly increased, and then reached the peak in next January ($Cr = 27.91\%$), February ($MA = 6.29$ mites/rat; $MI = 10.20$ mites/rat) and March ($P_M = 67.58\%$). From December to next March, all the infestation parameters maintained at a very high level (*Cr*: 18.14%–27.91%; P_M: 59.26%–67.58%; *MA*: 3.65–6.29; $MI = 6.15$–10.20), forming an obvious seasonal fluctuation pattern (Table 4, Fig. 6).

3.3. Correlation between infestations of R. brunneusculus with chiggers and climatic factors

Pearson's correlation analysis showed that the rainfall

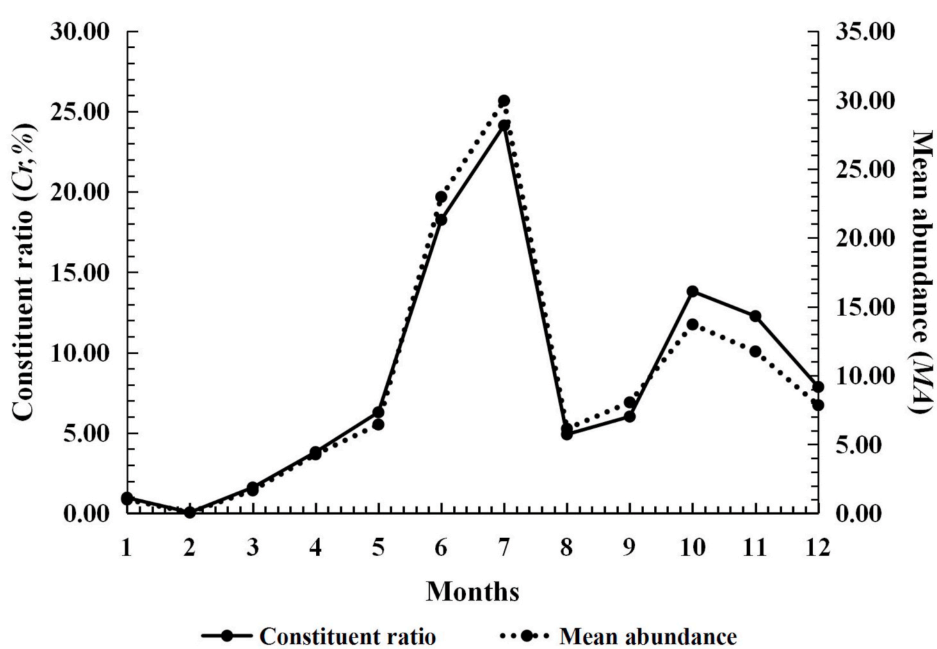

Fig. 4. Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. brunneusculus*) with *Leptotrombidium* (*L.*) *deliense* at Jingha, southern Yunnan of China (April 2016–March 2017).

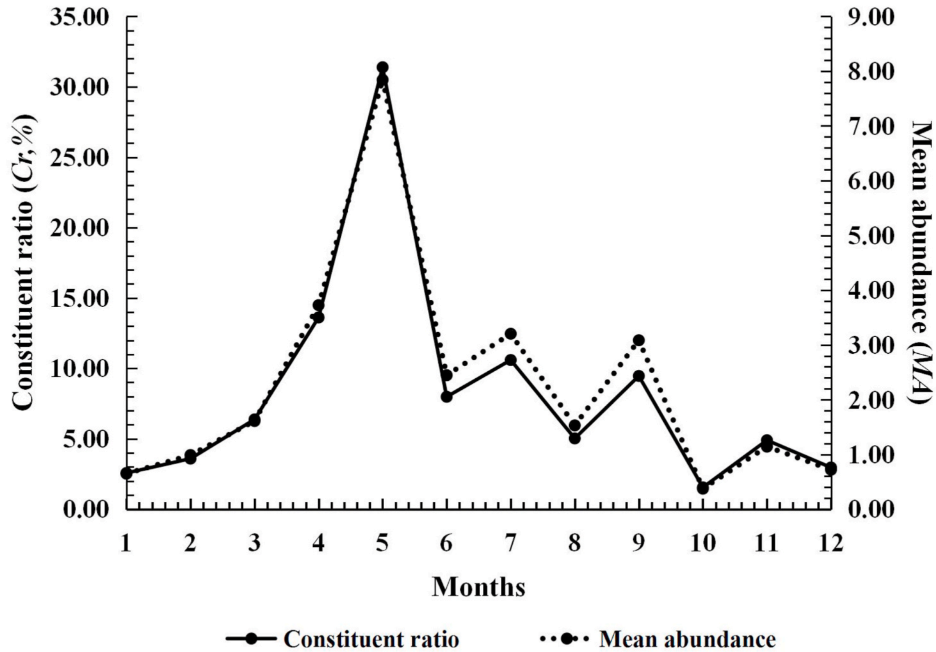

Fig. 5. Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. brunneusculus*) with *Walchia* (*W.*) *turmalis* at Jingha, southern Yunnan of China (April 2016–March 2017).

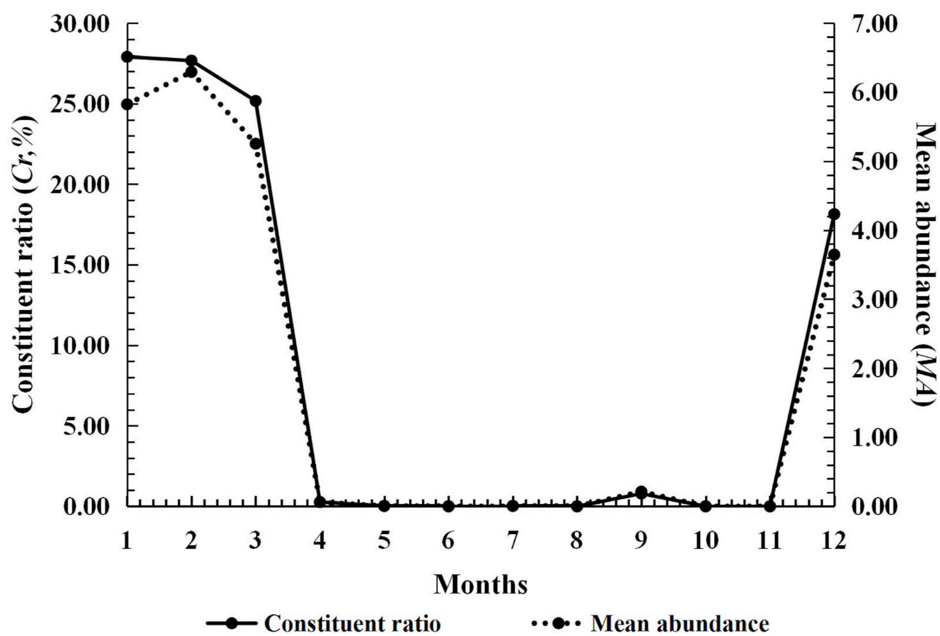

Fig. 6. Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. brunneusculus*) with *Leptotrombidium* (*L.*) *scutellare* at Jingha, southern Yunnan of China (April 2016–March 2017).

(precipitation) was positively correlated with all the infestation parameters (P_M, *MA* and *MI*) of *A. indica* (*r:* 0.776 for P_M, 0.815 for *MA* and 0.812 for *MI*; $P < 0.01$), but negatively correlated with the P_M of *L.* (*L.*) *scutellare* ($r = -0.596$, $P < 0.05$). The average temperature was positively correlated with all the infestation parameters (P_M, *MA* and *MI*) of *W.* (*W.*) *turmalis* (*r:* 0.691 for P_M, 0.669 for *MA* and 0.640 for *MI*; $P < 0.05$) and the mean intensity (*MI*) of *A. indica* ($r = 0.579$, $P < 0.05$), but negatively correlated with all the infestation parameters (P_M, *MA* and *MI*) of *L.* (*L.*) *scutellare* (*r:* −0.734 for P_M, −0.725 for *MA* and −0.804 for *MI*; $P < 0.05$). Although a negative correlation existed between the average humidity and the *Cr* of *W.* (*W.*) *micropelta* ($r = -0.594$, $P < 0.05$), the humidity had little effect on the other 4 chigger species ($P > 0.05$) (Table 5).

4. Discussion

4.1. Species diversity and overall infestation of chiggers on the Southeast Asian house rat

There are more than 3,700 species of chigger mites widely distributed in the world and more than 400 species recorded in China (Li et al., 1997; Lv et al., 2019; Zhang et al., 2011). In the present study, a total of 102 chigger species were found on the Southeast Asian house rat (*R. brunneusculus*) with high overall infestations (Table 1). The 102

Table 5
Pearson's linear correlation analysis between infestations of *R. brunneusculus* with five main chigger species and climatic factors (temperature, humidity and rainfall) in 12 months at Jingha village in southern Yunnan of China (April 2016–March 2017).

species	Infestation index	Coefficient of Pearson's correlation: *r* (*P*)		
		Total rainfall (mm)	Average temperature (°C)	Average humidity (%)
W. (*W.*) *micropelta*	*Cr*	−0.002 (0.995)	0.354 (0.258)	−0.594* (0.042)
	P_M	0.373 (0.233)	0.502 (0.096)	−0.201 (0.530)
	MA	0.101 (0.754)	0.442 (0.151)	−0.541 (0.069)
	MI	0.062 (0.847)	0.417 (0.177)	−0.559 (0.059)
A. indica	*Cr*	0.802** (0.002)	0.503 (0.096)	0.309 (0.329)
	P_M	0.776** (0.003)	0.490 (0.105)	0.432 (0.161)
	MA	0.815** (0.001)	0.528 (0.078)	0.315 (0.319)
	MI	0.812** (0.001)	0.579* (0.049)	0.250 (0.432)
L. (*L.*) *deliense*	*Cr*	0.519 (0.084)	0.267 (0.402)	0.512 (0.089)
	P_M	0.508 (0.092)	0.400 (0.198)	0.475 (0.118)
	MA	0.562 (0.057)	0.318 (0.314)	0.492 (0.104)
	MI	0.557 (0.060)	0.339 (0.281)	0.467 (0.126)
W. (*W.*) *turmalis*	*Cr*	0.194 (0.546)	0.611* (0.035)	−0.327 (0.299)
	P_M	0.285 (0.370)	0.691* (0.013)	−0.253 (0.428)
	MA	0.246 (0.441)	0.669* (0.017)	−0.301 (0.342)
	MI	0.207 (0.519)	0.640* (0.025)	−0.324 (0.305)
L. (*L.*) *scutellare*	*Cr*	−0.563 (0.057)	−0.733** (0.007)	−0.223 (0.487)
	P_M	−0.596* (0.041)	−0.734** (0.007)	−0.195 (0.543)
	MA	−0.562 (0.057)	−0.725** (0.008)	−0.231 (0.470)
	MI	−0.646 (0.084)	−0.804* (0.016)	−0.117 (0.783)

Annotation: The figures in the Table represent the coefficients of Pearson's correlation (*r*), and the figures in the brackets stand for the probability of significance (*P*). *The correlation coefficients are of significance at 0.05 level (double tails). **The correlation coefficients are of significance at 0.01 level (double tails).

chigger species identified from such a single rat species at a localized area (Jingha village) are more than the chigger species recorded in some other provinces of China (e.g. 53 species in Fujian, 41 species in Hubei and 34 species in Sichuan) (Li et al., 2010; Wang and Liao, 1981; Yang and Liu, 2003), and even exceed all the chigger species in some countries (62 species in Nepal, 27 species in Afghanistan and 18 species in Poland) (Daniel et al., 2010; Daniel and Stekolnikov, 2009; Moniuszko and Mąkol, 2014; Peng et al., 2016). The result suggests that the Southeast Asian house rat has a great potential to harbor many chiggers with high species diversity. The overall infestation parameters of chiggers on Southeast Asian house rats showed a high level from May to October with a peak in July (Table 1, Fig. 1) and this is consistent with the fluctuation of scrub typhus in southern Yunnan (Yuan et al., 2018; Zhang, 2001). The abundant chiggers occurred in summer (July) may increase the risk of scrub typhus from the rats to human beings through the biting activity of chiggers.

4.2. Five main species of chigger mites on the Southeast Asian house rat

Of the 102 chigger species, five of them were the most abundant on *R. brunneusculus* and they are *Walchia* (*W.*) *micropelta*, *A. indica*, *L.* (*L.*) *deliense*, *W* (*W.*) *turmalis* and *L.* (*L.*). *scutellare* (Table 3). Of the five main chigger species, *L.* (*L.*) *deliense* is the most powerful vector of scrub typhus and *L.* (*L.*) *scutellare* is the second major vector in China (Li et al., 1997; Lv et al., 2018; Su et al., 2012; Wu et al., 2013). Besides transmitting scrub typhus, *L.* (*L.*) *scutellare* is also suspected to potentially transmit hemorrhagic fever with renal syndrome (HFRS) (Li et al., 1997; Santibáñez et al., 2015). *Leptotrombidium* (*L.*) *deliense* and *L.* (*L.*) *scutellare* often invade and sting humans and it is very easy for them to transmit the diseases from rats to humans (Li et al., 2005; Santibáñez et al., 2015; Wu, 2005). *Ascoschoengastia indica* is a potential vector of scrub typhus and it can carry *O. tsutsugamushi* (Chaisiri et al., 2019; Tilak and Kunte, 2019; Wu et al., 2013). The abundant *L.* (*L.*) *deliense*, *L.* (*L.*) *scutellare* and *A. indica* found on *R. brunneusculus* further increase the risk of scrub typhus from the rats to humans. To date there has been no evidence to show that *W.* (*W.*) *micropelta* and *W.* (*W.*) *turmalis* can be effective vectors of scrub typhus and some other zoonoses. The medical importance of abundant *W.* (*W.*) *micropelta* and *W.* (*W.*) *turmalis* found on *R. brunneusculus* remains unclear and further researches may be needed, including the isolation of the relevant pathogens from the mites.

4.3. Seasonal fluctuation of five main species of chigger mites

It is necessary to study the seasonal fluctuation pattern of chigger mites, which often influences the prevalence of scrub typhus (Candasamy et al., 2016; Li et al., 1997; Lv et al., 2019). In the present study, *Cr* and *MA* were selected as two effective parameters to depict the seasonal fluctuation curves of five main species of chiggers on the Southeast Asian house rat. The *Cr* is to reflect the percentage of each mite species in the mite community, and *MA* is to reflect the mites per examined rat (Peng et al., 2018). It is a good way to use these two parameters to illustrate the seasonal fluctuation patterns of chiggers and some other ectoparasites (Chen, 1980; Frances et al., 1999; Oorebeek and Kleindorfer, 2008).

The five main chigger species had their own seasonal fluctuation patterns. Before the present study, some previous investigations had reported the seasonal fluctuations of *L.* (*L.*) *deliense*, *L.* (*L.*) *scutellare* and *A. indica* in some other provinces of China and some other countries (Frances et al., 1999; Kim et al., 2019; Li et al., 1997; Noda et al., 1996, 2013; Wu et al., 2013), but no literature was on the seasonal fluctuations of *W.* (*W.*) *micropelta* and *W.* (*W.*) *turmalis*. In the present study, the *Cr*, *MA* and *MI* of *W.* (*W.*) *micropelta* were relatively high from March to July with a slight peak in May and the mite had an irregular seasonal fluctuation without an obvious peak (Fig. 2). A negative linear correlation existed between average humidity and the *Cr* of *W.* (*W.*) *micropelta* ($P < 0.05$) and this suggests that the higher the humidity, the less the chigger mites. The result may imply that the high humidity may inhibit the survival and reproduction of *W.* (*W.*) *micropelta*. The result, however, is not consistent with the general opinions. According to the general opinions, the high humidity with much water vapor in the air is believed to be beneficial to the survival of most chigger mites (Clopton and Gold, 1993; Li et al., 1997). The seasonal fluctuation of *W.* (*W.*) *turmalis* belonged to spring type with a very prominent peak in May (Fig. 5). The average temperature was positively correlated with all the infestation parameters (P_M, *MA* and *MI*) of *W.* (*W.*) *turmalis* ($P < 0.05$). The positive correlation suggests that warm temperature may be beneficial to the survival, development and reproduction of *W.* (*W.*) *turmalis*, and this is in accordance with the situation of most chigger mites (Chaisiri et al., 2019; Clopton and Gold, 1993; Li et al., 1997; Santibáñez et al., 2015).

Different from *W.* (*W.*) *micropelta*, *A. indica* showed an obvious seasonal fluctuation though it could be found throughout the year. The seasonal fluctuation of *A. indica* seems to be spring-summer type. The mite show a small peak in May and the highest peak in August (Fig. 3). Some previous investigations from some other provinces of China showed that the seasonal peak of *A. indica* was in summer and autumn. The mite increased after May, decreased from August on and reached the lowest level (even no mites) in winter (Li et al., 1997). The seasonal fluctuation of *A. indica* in the present study is consistent with the previous records. In the present study, the rainfall (precipitation) was positively correlated with all the infestation parameters of *A. indica* ($P < 0.01$), and the average temperature was positively correlated with the *MA* of *A. indica* ($P < 0.05$). In the investigated site, it is hot and humid in summer with high temperature and rich rainfall (Lv et al., 2019; Sun et al., 2000). The positive correlations suggest that the high temperature with rich rainfall in summer may be beneficial to the survival and reproduction of *A. indica*.

As the most powerful vector of scrub typhus in China, *Leptotrombidium* (*L.*) *deliense* is believed to be the most major chigger species in the areas south of the Yangtz River (Lv et al., 2018; Su et al., 2012; Wu et al., 2013). In some regions of south Asian and southeast Asia, *L. deliense* is also a main vector of scrub typhus (Elliott et al., 2019; Santibáñez et al., 2015; Tilak and Kunte, 2019). Although the seasonal fluctuation curves of *L.* (*L.*) *deliense* vary in different geographical regions because of different latitude zones, altitudes and climates (Frances et al., 1999; Gentry et al., 1963; Lien et al., 1976), the mite usually has a preference to hot and humid weather (Candasamy et al., 2016; Frances et al., 1999; Li et al., 1997; Yuan et al., 2003; Zhang, 1994). In the laboratory, the warm temperature (18–28 °C) with high relative humidity (95–100%) is beneficial to the survival, development and reproduction of the mite (Li et al., 1997; Lv et al., 2018). In the present study, *L.* (*L.*) *deliense* was very abundant in summer, and the seasonal fluctuation of *L.* (*L.*) *deliense* belonged to summer-autumn type with the highest peak (the first peak) in July and the second small peak in October (Fig. 4). The result is similar to that in some other provinces of China and some other countries, in which the seasonal peak of *L.* (*L.*) *deliense* population often appeared in summer or/and autumn (Candasamy et al., 2016; Frances et al., 1999; Li et al., 1997; Yuan et al., 2003; Zhang, 1994).

Leptotrombidium (*L.*) *scutellare* is also a powerful vector of scrub typhus in China, which is second only to *L.* (*L.*) *deliense* (Li et al., 1997; Lv et al., 2018; Su et al., 2012). As a main chigger species in cold seasons, the seasonal fluctuation pattern of *L.* (*L.*) *scutellare* was opposite to that of *L.* (*L.*) *deliense*. The mite mainly appeared in winter and spring (from December to March) and its seasonal peak of *Cr* and *MA* was from January to February (Fig. 6). The result is consistent with that in some other provinces of China and some other countries (Choi et al., 2018; Li et al., 1997; Liu et al., 2004; Noda et al., 2013; Park and Shin, 2016; Pham et al., 1999; Wu et al., 2000; Yuan et al., 2003). Although the seasonal fluctuation curves of *L.* (*L.*) *scutellare* vary in different provinces of China, the basic pattern belongs to the autumn-winter type with the seasonal peak in cold seasons, late autumn and winter (Bang et al., 2008; Elliott et al., 2019; Santibáñez et al., 2015; Tilak and Kunte, 2019). The average temperature was negatively correlated with all the infestation parameters of *L.* (*L.*) *scutellare* ($P < 0.05$). The abundance of *L.* (*L.*) *scutellare* obviously increased with the decrease of temperature (Table 5). In addition, the rainfall (precipitation) was also negatively correlated with the P_M of *L.* (*L.*) *scutellare* ($P < 0.05$). The results suggest that the high temperature with rich rainfall is not good for the survival, development and reproduction of *L.* (*L.*) *scutellare* and the mite prefers a relatively cold and dry season to a hot and humid one.

5. Conclusions

The Southeast Asian house rat (*R. brunneusculus*) in southern Yunnan of China can harbor a variety of chigger species with high infestation. The five main chigger species on the rats are *W.* (*W.*) *micropelta*, *A. indica*, *L.* (*L.*) *deliense*, *W* (*W.*) *turmalis* and *L.* (*L.*) *scutellare*, and they have different patterns of seasonal fluctuation. The seasonal fluctuation of the vector *L. deliense* belongs to summer-autumn type with the highest peak in July, and the vector *L. scutellare* mainly appears in winter and spring with the peak from January to February. Temperature and rainfall (precipitation) are two key factors which influence the seasonal fluctuation of chigger mites.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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