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▣ ORIGINAL ARTICLE

Infestation and Related Ecology of Chigger Mites on the Asian House Rat (*Rattus tanezumi*) in Yunnan Province, Southwest China

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Abstract: This paper is to illustrate the infestation and related ecological characteristics of chigger mites on the Asian house rat (*Rattus tanezumi*). A total of 17,221 chigger mites were collected from 2,761 *R. tanezumi* rats, and then identified as 131 species and 19 genera in 2 families. *Leptotrombidium deliense*, the most powerful vector of scrub typhus in China, was the first major dominant species on *R. tanezumi*. All the dominant mite species were of an aggregated distribution among different individuals of *R. tanezumi*. The species composition and infestations of chiggers on *R. tanezumi* varied along different geographical regions, habitats and altitudes. The species-abundance distribution of the chigger mite community was successfully fitted and the theoretical curve equation was $\hat{S}(R) = 37e^{-(0.28R)^2}$. The total chigger species on *R. tanezumi* were estimated to be 199 species or 234 species, and this further suggested that *R. tanezumi* has a great potential to harbor abundant species of chigger mites. The results of the species-plot relationship indicated that the chig-ger mite community on *R. tanezumi* in Yunnan was an uneven community with very high heterogeneity. Wide geographi-cal regions with large host samples are recommended in the investigations of chigger mites.

Key words: *Rattus tanezumi*, Acari, chigger mite, rodent, Yunnan, China

INTRODUCTION

Chigger mites are a group of arthropods, and they are also known as trombiculid mites, tsutsugamushi mites, sand mites, grass itch mites, scrub itch mites, or harvest mites. In zoological taxonomy, chigger mites belong to 2 families (Trombiculidae and Leeuwenhoekiidae) of the order Acariformes in the subclass Acari of the class Arachnida [1,2]. There are over 3,700 species of chigger mites recorded worldwide and more than 510 species documented in China [3-5]. The life cycle of chigger mites was complicated with 7 stages, in which only the larvae are the ectoparasites on the body surface of some other animals [2,6,7]. The larvae of chigger mites are often called "chiggers". Chiggers have extensive hosts, including mammals, aves (birds), reptiles, amphibians, and even some other arthropods. Rodents and some other small mammals (e.g., insectivores and tree shrew) are the most common hosts of chiggers [6,8]. Chiggers are a group of important ectoparasites and medical arthropods, and the stinging of chiggers on human skin can directly lead to dermatitis. More importantly, some species of chigger mites (chiggers) are the exclusive vector of scrub typhus caused by the agent *Orientia tsutsugamushi* [6,9]. Besides transmitting the pathogen of scrub typhus (*O. tsutsugamushi*), chigger mites have also been proved to be the potential vector of hemorrhagic fever with renal syndrome (HFRS, also called epidemic hemorrhagic fever EHF) which was caused by hantavirus [10,11]. Yunnan Province (394,100 km^2) is located in the southwest of China with a high biodiversity of animals and plants, and it is also an important focus of scrub typhus, HFRS, and some other zoonoses [7,12,13].

The Asian house rat, *Rattus tanezumi* Temminck 1844, is a very common rodent species in Yunnan Province, and it is often called *R. flavipectus* (Milne-Edwards 1871) in China [14,15]. As a major commensal rodent species living around the residential areas of humans, the Asian house rat (*R. tanezumi* or *R. flavipectus*) is of great importance in both agriculture and medicine. Besides destroying crops as an agriculture pest, *R. tanezumi* is an important infectious source and reservoir host of

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many zoonoses, including plague, HFRS, scrub typhus (tsutsugamushi disease), endemic typhus (murine typhus), leptospirosis, etc. [14-17]. *Rattus tanezumi* often harbor a variety of ectoparasites, such as chigger mites (chiggers), gamasid mites, fleas, and sucking lice. The frequent occurrence of chigger mites on *R. tanezumi* makes it easy to transmit scrub typhus and HFRS from rodents to humans. Therefore, it is of medical importance to study the chigger mites on *R. tanezumi*.

A previous study from Guo et al. [14] made a preliminary analysis of chigger communities associated with *R. flavipectus* in 6 counties of Yunnan Province between 2001 and 2002. The field investigation in the previous study, however, was confined to only 6 counties, and it did not cover different geographical orientations and locations of the province [14]. On the basis of the previous study, our research group successively made a series of investigations from 2001 to 2015 and accumulated abundant original data. The investigated sites in the present study covered different geographical orientations of Yunnan Province with 33 investigated counties. To make full use of the abundant original data, the present study adopted some ideas and strategies of "data mining" [18,19], and made a systematic analysis on the infestation and some ecological issues of chigger mites on the Asian house rat (*R. tanezumi* or *R. flavipectus*), a single species of rodent. Besides the infestation analysis, the ecological issues in the present study involved the species diversity (species richness), species composition, species abundance distribution, and species-plot relationship of chigger mites on the pest *R. tanezumi*. The present study is an attempt to enrich the knowledge about chigger mites and provide more valuable information for the future study of chigger mites and some other ectoparasites.

MATERIALS AND METHODS

Ethics statement

The capture of animal hosts in the study was officially approved by the local wildlife affairs authority. The use of animals for the research (including rodent euthanasia) was also formally approved by the Animal Ethics Committee of Dali University, which followed the international standards of animal euthanasia, 2013 AVMA guidelines [20]. The approval number of animal ethics was DLDXLL2020-1104.

Field investigation

The original data came from a long-term accumulated field investigation in 34 investigation sites selected from 33 counties of Yunnan Province, southwest China between 2001 and 2015. The 34 investigation sites include Gongshan, Jianchuan, Yulong (Lijiang), Xianggelila, Lanping, Weixi, Deqin, Gengma, Cangyuan (Lincang), Yongde, Ninger, Yuanjiang, Dali, Binchuan, Mengzi, Qiaojia, Wenshan, Suijiang, Fuyuan, Luliang, Longchuan, Lianghe, Ruili, Jinghong, Hekou, Maguan, Menghai, Simao (Puer), Lushui, Fugong, Jinping, Mengla, Jingha, and Qiubei. Of the 34 investigation sites, Jingha and Jinghong sites belong to the same county, namely Jinghong County (Fig. 1).

Collection and identification of animal hosts and chigger mites

A stratified investigation was made in different geographical orientations, landscapes, altitude gradients and habitats. In each investigation site with different geographical orientations, landscapes and altitudes, mousetraps were placed in 2 types of habitats (the indoor habitats and the outdoor habitats) to capture rodents and some other sympatric small mammals with rodents (insectivores and tree shrews, for example) in the evening and then collected in the next morning. The indoor habitats involve houses, barns and stables, etc. and the outdoor habitats include paddy fields, corn fields, bush habitats and woodlands, etc. [6,21]. Each captured animal host (rodents and some other small mammals) was put into a white cloth bag and then brought to a temporary laboratory where the larvae of chigger mites (chiggers) were collected. The chiggers were mainly collected from the host's 2 auricles and external auditory canals, the most common attached sites of chiggers. The collected chiggers were preserved in vials with 70% ethanol [6]. Hoyer's medium was used to mount chiggers onto a glass slide. After a dehydrated and transparent process, the mounted specimens of chiggers were finally identified into species under a Leica DM 3000 LED microscope (Company name: Leica; city/town: Wetzlar; country: Germany) [11,22]. After the identifications of animal hosts and chigger mites, all the Asian house rats (*R. tanezumi*), together with all the chiggers on them, were selected as the target of the present study.

Infestation statistics of chigger mites

The constituent ratio (C_r) was used to calculate the proportion of each species of chigger mites (chiggers) on the Asian house rat, *R. tazemuzi*. The prevalence (P_m), mean abundance (MA) and mean intensity (MI) were used to analyze the infestations of *R. tazemuzi* with the mites [7,14].

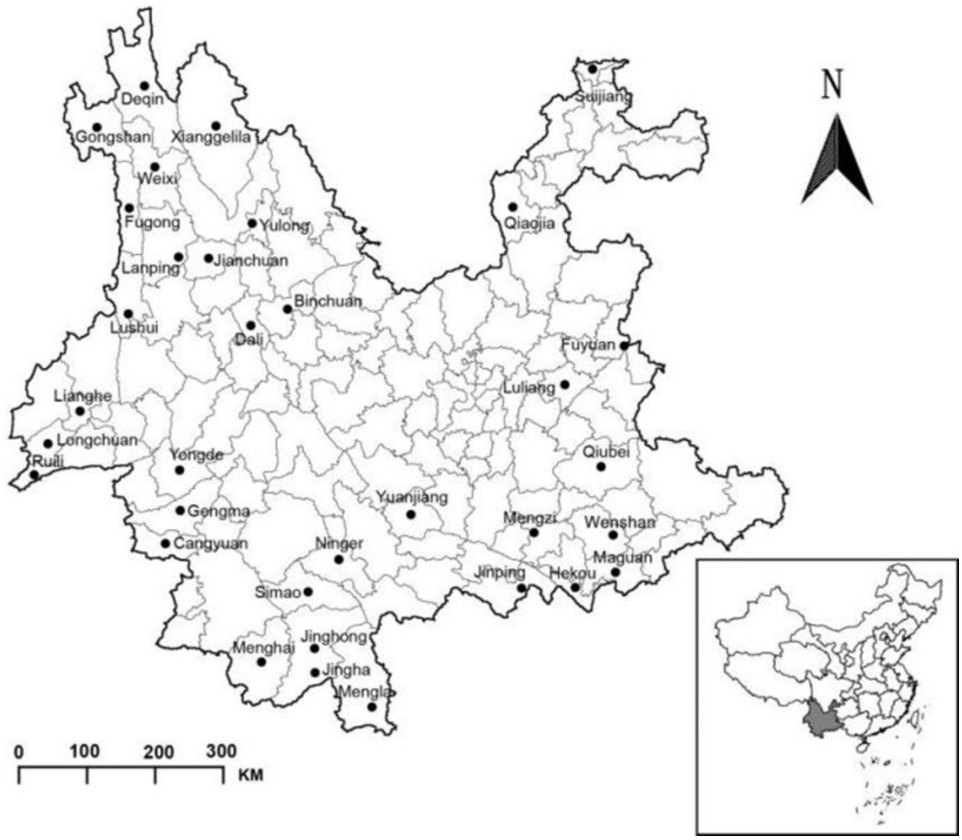

Fig. 1. The 34 investigation sites selected from 33 counties of Yunnan Province of southwest China (2001-2015).

$$C_r = \frac{N_i}{N}\times 100\%;\ P_m = \frac{H_i}{H}\times 100\%;\ MA = \frac{N_i}{H};\ MI = \frac{N_i}{H_i}$$

In the above formulae, N_i represents the individuals of chigger species *i* on the Asian house rat (*R. tazemuzi*), *N* the total individuals of all the species of chiggers on the hosts (*R. tanezumi*), H_i the individuals of infested hosts and *H* the total individuals of examined hosts.

Ecological statistics of chigger mite community

The richness (*S*), Shannon-Wiener's diversity index (*H*'), Cody index (β_C) and Jaccard index (C_J) were used to reflect the basic characteristics of chigger mite community on *R. tazemuzi* [11,23].

$$S=\sum S_i;\ H'=-\sum_{i=1}^{s}\left(\frac{N_i}{N}\right)\ln\left(\frac{N_i}{N}\right);\ \beta_C=\frac{g(H)+l(H)}{2}=\frac{a+b-2c}{2};\ C_J=\frac{c}{a+b-c}$$

In the above formulae, S_i represents chigger mite species i and N_i/N is the same as that in the former formulae. The a stands for the species of chigger mites in the environmental gradient A, *b* the species in the gradient B, and *c* the common species in both A and B. The $g(H)$ is the species increased along the environmental gradients and $l(H)$ is the species lost along the gradients. The value of C_J ranges from 0 to 1.

Spatial distribution patterns of chigger mites

The patchiness index (m^*/m), together with the *k* index from the negative binomial distribution, was used to measure the spatial distribution patterns of the dominant species of chigger mites among different individuals of *R. tanezumi* rats [14,15,24].

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

In the above formulae, m and σ^2 stands for the mean and variance of chigger mites respectively. When $m^*/m<1$ and $k<0$, the spatial distribution pattern is determined as uniform (or even) distribution; $m^*/m=1$ and $k\rightarrow\infty$ or $k>8$ the random distribution; and $m^*/m>1$ and $k>0$ the aggregated distribution [25].

Table 1. The contingency table for measuring the interspecific association between any 2 species of chigger mites on the Asian house rats (*Rattus tanezumi*)

		Species X		
		+ (yes)	– (no)	Total
Species Y	+ (yes)	*a*	*b*	*a*+*b*
	– (no)	*c*	*d*	*c*+*d*
Total		*a*+*c*	*b*+*d*	*n*

Analysis of interspecific association

Based on the establishment of a contingency table, the association coefficient (V) was used to measure the interspecific association between any 2 species of chigger mites on *R. tanezumi* rats. Chi-square test was used for testing the statistical significance of V [26]. In the contingency table, "+" and "–" mean the presence and absence of the mite species X or Y, and the *a*, *b*, *c*, and *d* represent the number of the hosts (host individuals), *R. tanezumi* rats (Table 1).

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

When $0 < V \leq 1$ ($P < 0.05$), the interspecific relationship is determined as the positive association, and when $-1 \leq V < 0$ ($P < 0.05$), the negative association [14].

Species abundance distribution and species-plot relationship

To illustrate the species abundance distribution of the chigger mite community on *R. tanezumi*, a semi-logarithmic rectangular system was established. The X-axis scaled with log-intervals ($\log_3 N$) was marked with the individuals of chigger mites and the Y-axis with arithmetic scales was marked with the species of chigger mites. Preston's lognormal model was used to fit the theoretical curve of species abundance distribution as the following formula [14,27].

$$\hat{S}(R) = S_0 e^{-[a(R-R_0)]^2} \quad (e{=}2.71828\ldots)$$

Where $\hat{S}(R)$ stands for the theoretical species number in R-th log interval, R the log interval R, R_0 the log interval corresponding to the highest point in the actual curve of species abundance, S_0 the mite species at R_0 log interval, and α is the spread constant of the distribution. The value of α was determined according to the best determination coefficient (R^2) in statistics [28].

$$R^2 = 1 - \frac{\sum_{R=0}^{m}[S(R) - \hat{S}(R)]^2}{\sum_{R=0}^{m}[S(R) - \bar{S}(R)]^2}; \quad \bar{S}(R) = \frac{1}{m}\sum_{R=0}^{m} S(R)$$

Where $S(R)$ is the actual chigger mite species in R-th log interval and m is the number of log intervals in the fitting of theoretical curve.

In the study of the species-plot relationship of the chigger mite community on *R. tanezumi*, the X-axis was marked with the number of "sampling plots", while the Y-axis with the number of chigger mite species [25]. The "sampling plots" are actually the individuals of the rat hosts (*R. tanezumi*) in the present study.

Estimation of total species of chigger mites

Two types of estimation methods were used to estimate the total species of chigger mites on the Asian house rat (*R. tanezumi*) in Yunnan. One type of estimation method is Chao 1 formula (S^*) which was based on the collected rare species in the community [29]. The other type of estimation method (S_T) is based on Preston's lognormal model for the species abundance distribution [28,30].

$$S^* = S_{obs} + \frac{a^2}{2b}; \quad S_T = \left(S_0\sqrt{\pi}\right)/\alpha$$

In the estimation type of Chao 1 formula, S^* = the total species of chigger mites on the Asian house rat (*R. tanezumi*) estimated by Chao 1 formula, S_{obs} = the total species number of chigger mites actually collected (observed) in the sampling investigation, a = the number of chigger mite species with only 1 individual collected, b = the number of chigger mite species with only 2 individuals collected. In the type of estimation method (S_T), S_T = the total species of chigger mites on the Asian house rat (*R. tanezumi*) estimated by the method based on Preston's lognormal model, π = 3.141592654, and S_0 and α are the same as those in the former formulae of species abundance distribution.

RESULTS

Species diversity and overall infestations of chigger mites on *Rattus tanezumi*

A total of 2,761 Asian house rats (*R. tanezumi*) were captured from 29 of 33 investigation counties. There were abundant species of chigger mites on *R. tanezumi* rats. From the body surface of 2,761 Asian house rats, 17,221 individuals of chigger mites (chiggers) were collected, and they were identified as 131 species with high species diversity. The 131 chigger mite species belong to 19 genera and 3 subfamilies (Trombiculinae, Gahrliepiinae and Leeuwenhoekiinae) in 2 families, Trombiculidae and Leeuwenhoekiidae (Table 2). Of the 17,221 individuals of chigger mites collected, 78.6% of them (C_r = 78.6%) came from the subfamily Trombiculinae and the mites in the genus *Leptotrombidium* accounted for 50.5% of the whole subfamily Trombiculinae. Based on the species diversity or species richness (S = 131), the Shannon-Wiener's diversity index (H') of the chigger mite community on *R. tanezumi* was calculated as 2.5 (H' = 2.5). The overall prevalence (P_m), overall mean abundance (*MA*) and overall mean intensity (*MI*) of the Asian house rats (*R. tanezumi*) with chigger mites were 20.9% (P_m = 20.9%), 6.2 mites per examined rat (*MA* = 6.2 mites/rat) and 29.8 mites per infested rat (*MI* = 29.8 mites/rat) respectively.

Infestations and spatial distribution patterns of dominant species of chigger mites

Of 131 species of chigger mites collected from *R. tanezumi*, the constituent ratio (C_r) of 4 dominant mite species accounted for 67.7% of the total mites. The 4 dominant species of chigger mites are *Leptotrombidium deliense* (Walch, 1922), *Ascoschoengastia indica* (Hirst, 1915), *Walchia ewingi* (Fuller, 1949) and *W. micropelta* (Traub et Evans, 1957). Of the 4 dominant mite species, *L. deliense* was the first dominant species with the highest constituent ratio (C_r = 27.2%), and *A. indica* came next (C_r = 26.0%). *Leptotrombidium deliense* had the highest prevalence (P_m = 6.3%) and mean abundance (*MA* = 1.7 mites/rat) while *A. indica* had the highest mean intensity (*MI* = 35.6 mites/rat) ($P < 0.05$) (Table 3). As shown in Table 3, the calculated values of m^*/m and k for the 4 dominant species of chigger mites were much higher than 0 or 1, the border values for the determination of aggregated distribution pattern, and therefore the spatial distribution patterns of *L. deliense*, *A. indica*, *W. ewingi* and *W. micropelta* were determined to be of aggregated distribution among different individuals of their rat host, *R. tanezumi*. In addition, the spatial distribution patterns of all the 131 species of chigger mites were also of aggregated distribution.

Comparison of mite infestations along different environmental gradients

Of the 29 counties where the rats (*R. tanezumi*) were captured, the infestations of the rats with chiggers were different in different counties, habitats, and altitude gradients. The majority of chigger mites were collected from 4 counties (Jinghong, Yuanjiang, Longchuan, and Lushui) with 84.3% of total constituent ratio (C_r = 84.3%). The prevalence of the mites was highest in Jinping County in the southeast of Yunnan (P_m = 60.9%), and the mean abundance (*MA*) and mean intensity (*MI*) were highest in Jinghong county in the south of Yunnan (*MA* = 21.9 mites/rat, *MI* = 50.6 mites/rat). The differences of mite infestations (P_m, *MA* and *MI*) in different counties were statistically significant ($P < 0.05$).

The species diversity (species richness) and infestations of chiggers on *R. tanezumi* in the outdoor habitat (S = 114 species, P_m = 27.2%, *MA* = 10.1 mites/rat, *MI* = 37.2 mites/rat) were much higher than those in the indoor habitat (S = 71 species, P_m = 15.3%, *MA* = 2.8 mites/rat, *MI* = 18.4 mites/rat) ($P < 0.05$) (Table 4). The mite species similarity between the indoor and outdoor habitats was low with a low value of Jaccard index: $C_J = 54/(71 + 114 - 54) = 0.4$.

Of 2,761 Asian house rats (*R. tanezumi*) captured, 2013 rats had altitude records and 748 rats had no altitude records. The 2013 *R. tanezumi* rats with altitude records were used in the analysis of the present study. The overall infestation prevalence (P_m) and overall mean abundance (*MA*) were highest at the lowest altitude gradient (< 500 m), but the overall mean intensity (*MI*) was highest at the highest altitude gradient (> 2,499 m). Although overall infestations (P_m, *MA* and *MI*) were different along 6 altitude gradients, only the differences of prevalence (P_m) and mean abundance (*MA*) were of statistical significance ($P < 0.05$). Between the altitude "500-999 m" and "> 2,499 m", the species richness (species diversity) of chigger mite community on *R. tanezumi* showed a gradually decreased tendency with the increase of altitude gradients (Table 5; Fig. 2). As shown in Fig. 3, the curves of Cody index (β_C) and Jaccard index (C_J) showed a parabolic tendency along the altitude gradients. The species similarity of chigger mites was very low along different altitude gradients with a very low value of Jaccard indexes ($C_J < 0.31$).

Interspecific association between dominant mite species

According to the association coefficient (V), a slightly positive association existed between the dominant species of chigger mites *W. micropelta* and *A. indica* ($V = 0.18$, $\chi^2 = 6.625$, $P < 0.05$), as well as between *L. deliense* and *A. indica* ($V = 0.12$, $\chi^2 = 8.437$, $P < 0.05$) on the rat host *R. tanezumi* (Table 6).

Table 2. Chigger mites collected and identified from the Asian house rats (*Rattus tanezumi*) in Yunnan Province, southwest China between 2001 and 2015

Taxonomic taxa of chigger mites	Collected species and individuals of chigger mites (The figures in brackets are collected individuals of each mite species)
Family Trombiculidae	17,195 individuals, 129 species, 17 genera, 2 subfamilies
Subfamily Trombiculinae	13,537 individuals, 97 species, 14 genera
Genus *Leptotrombidium*	6,836 individuals, 54 species
	Leptotrombidium scutellare (52); *L. sinicum* (8); *L. eothenomydis* (14); *L. hiemale* (1); *L. cricethrionis* or *L. rusticum* (115); *L. shuqui* (1); *L. Wangi* (11); *L. yongshengense* (65); *L. densipunctatum* (36); *L. yui* (3); *L. xiaguanense* (4); *L. deliense* (4,685); *L. imphalum* (699); *L. rubellum* (663); *L. gongshanense* (78); *L. spicanisetum* (21); *L. yuebeinse* (5); *L. cuonae* (5); *L. deplanoscutum* (53); *L. lianghense* (8); *L. quadrifurcatum* (1); *L. wenense* or *L. kaohuense* (6); *L. xiaowei* (19); *L. sheshui* (35); *L. chuanxi* (1); *L. qujingense* (26); *L. dianchi* (4); *L. jinmai* (1); *L. akamushi* (51) ; *L. alpinum* (8); *L. pallidum* (5); *L. robustisetum* (4); *L. biluoxueshanense* (5); *L. kawamurai* (2); *L. bawangense* (3); *L. dongluoense* (1); *L. dichotogalium* (2); *L. apodemi* (1); *L. hupeicum* (1); *L. kiangsuense* (1); *L. intermedium* (1); *L. longchuanense* (11); *L. rectanguloscutum* (2); *L. qiui* (1); *L. filasensillum* (1); *L. yigongense* (2); *L. bishanense* (1); *L. kitasatoi* (1); *L. ejingshanense* (2); *L. baoshui* (1); *L. saltuosum* (1); *L. guzhangense* (1); *L. shuyui* (1); *L. turdicola* or *L. muntiaci* or *L. suense* (106)
Genus *Trombiculindus*	5 individuals, 3 species
	Trombiculindus nujiange (1); *T. hylomydis* (1); *T. yunnanus* (3)
Genus *Neotrombicula*	1 individual, 1 specie
	Neotrombicula wendai (1)
Genus *Chiroptella*	7 individuals, 1 specie
	Chiroptella anhuiensis (7)
Genus *Lorillatum*	42 individuals, 2 species
	Lorillatum flagellasensilla (39); *L. tungshihensis* (3)
Genus *Microtrombicula*	309 individual, 1 specie
	Microtrombicula munda (309)
Genus *Eutrombicula*	65 individuals, 2 species
	Eutrombicula hirsti (49); *E. wichmanni* (16)
Genus *Helenicula*	394 individuals, 12 species
	Helenicula abaensis (2); *H. aulacochaeta* (1); *H. comata* (1); *H. globularis* (16); *H. hsui* (72); *H. kohlsi* (7); *H. miyagawai* (23); *H. myospalacis* (8); *H. olsufjevi* (4); *H. rattihaikonga* (1); *H. simena* (220); *H. lanius* (39)
Genus *Doloisia*	6 individuals, 4 species
	Doloisia brachypus (3); *D. furcipelta* (1); *D. manipurensis* (1); *D. sinensis* (1)
Genus *Cheladonta*	1 individual, 1 specie
	Cheladonta micheneri (1)
Genus *Ascoschoengastia*	5,811 individuals, 8 species
	Ascoschoengastia audyi (3); *A. indica* (4,483); *A. leechi* (321); *A. menghaiensis* (766); *A. montana* (1); *A. petauristae* (23); *A. yunnanensis* (78); *A. yunwui* (136)
Genus *Walchiella*	49 individuals, 2 species
	Walchiella notiala (44); *W. yingjiangensis* (5)
Genus *Herpetacarus*	10 individuals, 5 species
	Herpetacarus aristoclavus (1); *H. hastoclavus* (1); *H. limon* (3); *H. spinosetosus* (3); *H. tengchongensis* (2)

(*Continued to the next page*)

Table 2. Continued

Taxonomic taxa of chigger mites	Collected species and individuals of chigger mites (The figures in brackets are collected individuals of each mite species)
Genus *Euschoengastia*	1 individual, 1 specie
	Euschoengastia alpina (1)
Subfamily Gahrliepiinae	3,658 individuals, 32 species, 3 genera
Genus *Walchia*	3,195 individuals, 15 species
	Walchia chinensis (358); *W. chuanica* (20); *W. enode* (32); *W. erana* (1); *W. ewingi* (1,477); *W. koi* (66); *W. latiscuta* (1); *W. micropelta* (1,022); *W. minuscuta* (12); *W. nanfangis* (7); *W. parapacifica* (13); *W. rustica* (7); *W. turmalis* (145); *W. xishaensis* (30); *W. zangnanica* (4)
Genus *Schoengastiella*	364 individuals, 2 species
	Schoengastiella himalayana (2); *S. ligula* (362)
Genus *Gahrliepia*	99 individuals, 15 species
	Gahrliepia agrariusia (1); *G. banyei* (2); *G. fimbriata* (17); *G. lamella* (4); *G. latiscutata* (3); *G. lengshui* (1); *G. longipedalis* (18); *G. madun* (1); *G. meridionalis* (2); *G. octosetosa* (3); *G. radiopunctata* (8); *G. silvatica* (3); *G. yangchenensis* (11); *G. yunnanensis* (3); *G. zhongwoi* (22)
Family Leeuwenhoekiidae	26 individuals, 2 species, 2 genera, 1 subfamily
Subfamily Leeuwenhoekiinae	26 individuals, 2 species, 2 genera
Genus *Odontacarus*	12 individuals, 1specie
	Odontacarus tetrasetosus (12)
Genus *Chatia*	14 individuals, 1 specie
	Chatia maoyi (14)
Total	17,221 individuals, 131 species, 19 genera, 3 subfamilies, 2 families

Species abundance distribution and total species estimation of chigger mites

Based on Preston's lognormal model, the species abundance distribution of the chigger mite community on *R. tanezumi* was successfully fitted by the lognormal distribution with $\alpha = 0.28$ and $R^2 = 0.93$. The theoretical curve equation was $\hat{S}(R) = 37e^{-(0.28R)^2}$ ($S_0 = 37$, $R_0 = 0$). The tendency of theoretical curve showed the chigger mite species gradually decreased with the increase of chigger mite individuals (Table 7; Fig. 4).

Two types of estimation methods were used to estimate the total species of chigger mites. Based on Preston's lognormal model for the fitting of species abundance distribution, the total species of chigger mites on *R. tanezumi* were estimated to be 234 species ($S_T = 234$), 103 species more than the actual collected 131 species.

Of the 131 chigger mite species found on *R. tanezumi*, 37 mite species were rare species with only 1 individual collected. Based on Chao 1 formula, the total species of chigger mites on *R. tanezumi* were estimated to be 199 species ($S^* = 199$), 68 species more than the actual collected 131 species.

Species-plot relationship

In the study of species-plot relationship of chigger mite community on the Asian house rat (*R. tanezumi*), the examined individuals of *R. tanezumi* were defined as the "sampling plots". In a rectangular coordinate system, the X-axis was scaled with the examined individuals of *R. tanezumi* (sampling plots) and the Y-axis scaled with the collected species of chigger mites, and then the curve of species-plot relationship was depicted. The curve tendency of the species-plot relationship revealed that the species of chiggers increased with the increase of the individual rats (*R. tanezumi*). When the examined rats (*R. tanezumi*) reached 2,761 individuals, the curve of the species-plot relationship continued to keep a "going-up" tendency without the appearance of a "platform stage" (Fig. 5).

DISCUSSION

General significance of the present study

The larvae of chigger mites (chiggers) are very common ectoparasites on rodents (rats, mice and voles) and some other small mammals (e.g., insectivores and tree shrews). Besides invading and stinging humans to cause dermatitis, chiggers are the exclusive transmitting vector of scrub typhus (tsutsugamushi disease) and they are also suspected to be the potential

Table 3. The infestations and spatial distribution patterns of dominant chigger mites on the Asian house rats (*Rattus tanezumi*) in Yunnan Province, southwest China from 2001 to 2015

Dominant species of chigger mites	Constituent ratios of chigger mites		Infestations of chigger mites			Measurement of spatial distribution patterns	
	Individuals	C_r (%)	P_m (%)	*MA*	*MI*	m^*/m	*k*
Leptotrombidium deliense	4,685	27.2	6.3	1.7	26.8	66.2	0.015>0
Ascoschoengastia indica	4,483	26.0	4.6	1.6	35.6	188.2	0.005>0
Walchia ewingi	1,477	8.6	2.6	0.5	20.8	630.6	0.002>0
Walchia micropelta	1,022	5.9	3.2	0.4	11.5	379.5	0.003>0
All the 131 mite species	17,221	100	20.9	6.2	29.8	36.1	0.285>0

Table 4. The overall infestations of chigger mites on *R. tanezumi* in different habitats in Yunnan Province, southwest China from 2001 to 2015

Habitats	Host individuals	Chigger mites			Infestations of chigger mites on *R. tanezumi*		
		S	Individuals	C_r (%)	P_m (%)	*MA*	*MI*
Indoor	1,466	71	4,140	24	15.3	2.8	18.4
Outdoor	1,295	114	13,081	76	27.2	10.1	37.2
Total	2,761	131	17,221	100	20.9	6.2	29.8

Table 5. The infestations of chigger mites on the rats (*R. tanezumi*) in different altitude gradients in Yunnan Province, southwest China from 2001 to 2015

Different altitude gradients		No. of *R. tanezumi*	Species and individuals of chiggers			Infestations of chiggers on *R. tanezumi*		
			S	Individuals	C_r (%)	P_m (%)	*MA*	*MI*
I	<500	222	26	2,590	22.2	46.4	11.7	25.1
II	500-999	1,154	63	7,411	63.6	18.6	6.4	34.5
III	1,000-1,499	327	57	923	7.9	22.3	2.8	12.6
IV	1,500-1,999	125	42	408	3.5	27.2	3.3	12.0
V	2,000-2,499	151	18	127	1.1	8.6	0.8	9.8
VI	>2,499	34	4	189	1.6	11.8	5.6	47.3
Total		2,013	120	11,648	100	22	5.8	26.4

There were 748 individuals of *R. tanezumi* without altitude records, which harbored 5,573 individuals of chigger mites.

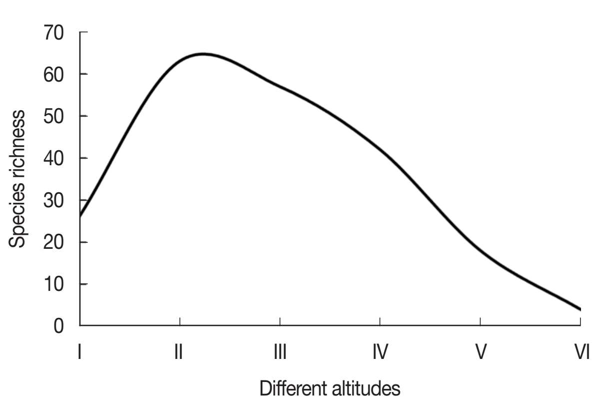

Fig. 2. The fluctuation of species richness (*S*) of chigger mite community along different altitudes.

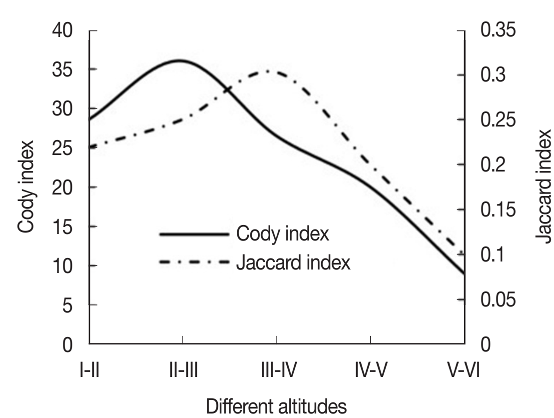

Fig. 3. The fluctuation of Jaccard index (C_J) and Cody index (β_C) of chigger mite community along different altitudes.

Table 6. Interspecific association between 2 dominant species of chigger mites on *R. tanezumi*

		Walchia micropelta			*Leptotrombidium deliense*		
		+	–	Total	+	–	Total
Ascoschoengastia indica	+	22	104	126	25	101	126
	–	67	2,568	2,635	150	2,485	2,635
	Total	89	2,672	2,761	175	2,586	2,761
Association coefficient			$V=0.18$			$V=0.12$	
Chi-square			$\chi^2=6.625$			$\chi^2=8.437$	
Significance			$P<0.05$			$P<0.05$	

Table 7. Theoretical curve fitting for species abundance distribution of chigger mite community on *R. tanezumi*

Log intervals	Individual ranges in each log interval	Midpoint values of each individual range	Actual chigger mites species	Theoretical chigger mites species
0	0-1	1	37	37.0
1	2-4	3	28	34.2
2	5-13	9	21	27.0
3	14-40	27	19	18.3
4	41-121	81	12	10.6
5	122-364	243	7	5.2
6	365-1,093	729	4	2.2
7	1,094-3,280	2,187	1	0.8
8	3,281-9,841	6,561	2	0.2

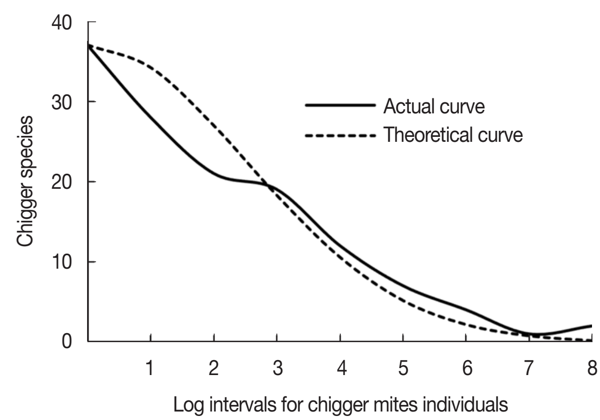

Fig. 4. Theoretical curve fitting for species abundance distribution of chigger mite community on *Rattus tanezumi*.

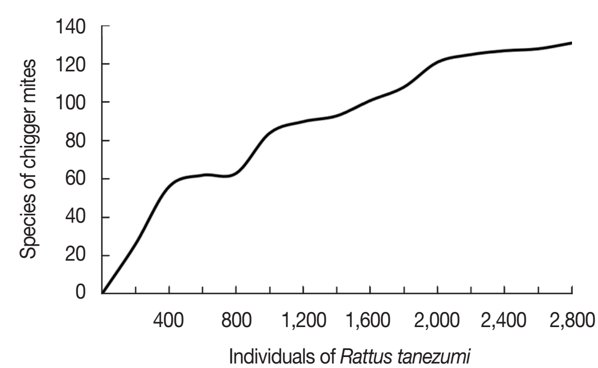

Fig. 5. The curve of species-plot relationship of the chigger mite community on *Rattus tanezumi*.

vector of hemorrhagic fever with renal syndrome (HFRS) [9-11]. As a very common sympatric pest with humans, the Asian house rat (*R. tanezumi*) is of great importance in both agriculture and medicine [14-17]. And therefore, it is of significance to study the chigger mites on *R. tanezumi* rats. Although there were some previous literatures involved the infestations, faunae and ecological distributions of chigger mites [6-9,14], few studies focused on the systematic analysis of chigger mites on a single species of rodent. And moreover, few previous reports came from the investigations in a wide geographical scope with large samples of animal hosts for a single species of rat. Located in southwest China, Yunnan Province is a big province with 394,100 km^2. The original data of the present study came from a long-term field investigation in 33 counties of Yunnan Province and the investigation covered different geographical orientations of the province, which is a systematic investigation in wide geographical regions. The examined Asian house rats (*R. tanezumi*) in the present study reached 2,761 rat individuals, a large sample of animal hosts. Based on such an extensive geographical investigation with large host samples, the results of the present study systematically revealed the infestation, species diversity and some ecological characteristics of chigger mites on *R. tanezumi* in Yunnan.

A previous study from Guo et al. [14] once made a preliminary analysis of chigger communities on *R. tanezumi* (*R. flavipectus*), but the field investigation was limited to only 6 counties. In the previous report, only 31 species of chigger mites were found on 451 *R. tanezumi* rats from 6 counties [14]. The investigated geographical scope in the present study reached

33 counties and the examined *R. tanezumi* rats (host samples) amounted to 2,761 individuals. The identified 131 species of chigger mites in the present study extremely exceeded the 31 species in the previous report. The results of the present study showed that the number of chigger mite species increased significantly with the increase of investigation sites, geographical scopes and host samples. The results further suggest that it is necessary to make an extensive geographical investigation with a large sample of animal hosts in field studies of chigger mites, so as to fully and thoroughly reveal the species composition and diversity of the mites.

Species diversity and overall infestations of chigger mites on *Rattus tanezumi*

A total of 17,221 chiggers, the larvae of chigger mites, were collected from 2,761 *R. tanezumi* rats in the present study and they were identified as 131 species of 19 genera under 2 families. The overall prevalence (P_m), overall mean abundance (*MA*) and overall mean intensity (*MI*) of chigger mites on *R. tanezumi* were 20.9%, 6.2 mites/rat and 29.8 mites/rat respectively. The 131 species of chigger mites identified from such a single rat species (*R. tanezumi*) indicated a very high species diversity (species richness) of the chigger mite community on the rat. The identified 131 species of chigger mites from *R. tanezumi*, a single species of rodent, obviously exceeded the total recorded species of chigger mites from different animal hosts in some other provinces of China [3,31,32] and even exceeded all the species of chigger mites recorded from a whole country [8,22, 33]. The results suggest that it is common for the Asian house rat (*R. tanezumi*) to be infested with chigger mites in Yunnan and *R. tanezumi* has a great potential to harbor abundant species of chigger mites. Besides the biological characteristics of *R. tanezumi* itself, the large samples of animal hosts (2,761 *R. tanezumi* rats) from a wide geographical scope (33 counties) are also important factors to lead to the high species diversity of chigger mites on such a single species of rat, *R. tanezumi*. Located in the southwest of China, Yunnan Province is known as the "kingdom of plants and animals" in China with abundant species of plants and animals because of its complex topography, ecological environments and varied climates [7,34]. In different geographical regions, the same species of host may harbor different species of chigger mites and some other ectoparasites [11,14] and this will increase the species diversity of chigger mites on *R. tanezumi*.

It has proved that there are 6 main vectors of scrub typhus in China and the 6 main vector species of chigger mites are *Leptotrombidium deliense*, *L. rubellum*, *L. scutellare*, *L. wenense* (*L. kaohuense*), *L. sialkotense* (*L. jishoum*) and *L. insularae* [35,36]. Besides the transmission of scrub typhus, *L. scutellare* can be the potential vector of hemorrhagic fever with renal syndrome [10]. Of the 6 main vectors of scrub typhus in China, 4 of them occurred on the Asian house rat (*R. tanezumi*) in the present study and the number of the 4 vector species accounted for 31.4% of the total 131 mite species. The 4 vector species of chigger mites on *R. tanezumi* rats found in Yunnan between 2001 and 2015 are *L. deliense*, *L. rubellum*, *L. scutellare* and *L. wenense* (*L. kaohuense*). The co-occurrence of the 4 vectors of scrub typhus with a high constituent ratio (C_r = 31.4%) on the Asian house rats (*R. tanezumi*) greatly increases the transmission risk of scrub typhus from the rats to humans. Before the present study, Lv et al. reported that *L. sialkotense* (*L. jishoum*), another important vector of scrub typhus in China, was found at Jingha village in southern Yunnan, which is the first record (new finding) of *L. sialkotense* in Yunnan [37]. The new finding of the chigger mite *L. sialkotense* at Jingha village was from a consecutive investigation from April 2016 to March 2017. At Jingha village, 186 *R. tanezumi* rats were examined and 8 individuals of *L. sialkotense* (*L. jishoum*) were identified from the rats [37]. Yunnan Province is an important focus of scrub typhus [7,12,13]. The results of the present study and the new finding from Jingha village suggest that the Asian house rat (*R. tanezumi*) can harbor the 5 vectors of scrub typhus, *L. deliense*, *L. rubellum*, *L. scutellare*, *L. wenense* (*L. kaohuense*) and *L. sialkotense* (*L. jishoum*), and this further increases the transmission risk of scrub typhus.

Dominant species of chigger mites on *Rattus tanezumi*

Of 131 species of chigger mites found on *R. tanezumi*, 4 of them were the dominant mite species with 67.7% of the total constituent ratio. The 4 dominant mite species are *L. deliense*, *A. indica*, *W. ewingi* and *W. micropelta*. Of the 4 dominant species of chigger mites on *R. tanezumi* rats, *L. deliense* was the first dominant mite species with highest constituent ratio (C_r = 27.2%) and relatively high infestations (P_m = 6.3, *MA* = 1.7 and *MI* = 26.8) and *A. indica* came next (Table 3). It has proved that *Leptotrombidium deliense* is the first major and most powerful vector of scrub typhus in China, and *A. indica* is a potential vector of the disease [1,36]. The high proportions and infestations of *L. deliense* and *A. indica* on *R. tanezumi*, further increase the transmission risk of scrub typhus from the rats to humans.

The measurement of spatial distribution pattern of a certain population is one of important issues in arthropod ecology. There are usually 3 types of spatial distribution patterns: uniform (or even) distribution, random distribution and aggregated distribution [25,26]. There are a variety of statistical methods to measure the spatial distribution pattern of a certain population, and the patchiness index (m^*/m) and k index (k) used in the present study are 2 of them [14,24]. The results showed that the 4 dominant species of chigger mites on *R. tanezumi* were all determined to be of aggregated distribution among different individuals of the rat host, which is consistent with many other parasites [15,25,38]. The aggregated distribution indicates that the infestations of the 4 dominant mite species are not even among different individuals of their host, *R. tanezumi*. Some *R. tanezumi* rats may harbor a large number of mites, forming a clump of mites on their body surface, while some other rats may have no or very few mites on their body surface. This aggregated distribution may be beneficial to the survival, mating, reproduction and defense of the parasites [15,25]. The aggregated distribution of chigger mites among different individuals of the Asian house rat (*R. tanezumi*) implies that the individuals of a certain rat species (e.g., *R. tanezumi*) with a clump of chigger mites on their body surface would have much higher risk of transmitting scrub typhus than the individuals of the same rat species without the mites. Although the aggregated distribution is a very common phenomenon in the field of ectoparasites including chigger mites [5,15,25,26,37,38], more researches are still needed to clarify its epidemiological significance associated with pest control.

The analysis of interspecific relationship between any 2 different species is an important issue in animal ecology [25,26]. The association coefficient (V) used in the present study is a simple way to measure the interspecific relationship between any 2 species. The results showed that a slightly positive association existed between the dominant species of chigger mites *W. micropelta* and *A. indica*, as well as between *L. deliense* and *A. indica*. The positive interspecific association suggests that 2 species of chigger mites have a tendency to co-occur on the body surface of their hosts [14,26].

Infestation fluctuation of chigger mites along different environmental gradients

The present study showed that the infestations of chigger mites on the Asian house rat (*R. tanezumi*) fluctuated along different environmental gradients, including different geographical regions, habitats and altitudes. In different geographical regions, the Asian house rat (*R. tanezumi*) had the highest prevalence with chigger mites (P_m = 60.9%) in Jinping County in the southeast of Yunnan, and it had the highest mean abundance (MA = 21.9) and mean intensity (MI = 50.6) with the mites in Jinghong County in the south of Yunnan. Located in the southwest of China, Yunnan Province is mainly a mountainous region with complex topography, different types of landscapes and habitats, and a variety of altitude gradients and climates. From the northwest to the southeast of Yunnan, the altitudes gradually decrease with the decrease of latitudes. The climate in the high altitude areas of northwest Yunnan is generally cold and dry, while the climate in the low altitude areas of southeast and south Yunnan is generally hot and humid with abundant rainfalls [1,5]. It is believed that warm and humid environments are generally beneficial to the development and reproduction of most chiggers [39,40]. The high temperature and humidity in the southeast and south Yunnan may be an important factor to lead to the high infestations of chigger mites on *R. tanezumi* in the regions.

The infestation differences of chigger mites on *R. tanezumi* occurred in different habitats. Jaccard index (C_J) was used in the present study to reveal the species similarity of chigger mites in different habitats. The result showed that the species similarity of chigger mites between the indoor and outdoor habitats was low with a low value of Jaccard index (C_J = 0.4). The species diversity (species richness) and infestations of chigger mites on *R. tanezumi* in the outdoor habitat (S = 114 species, P_m = 27.2%, MA = 10.1 mites/rat, MI = 37.2 mites/rat) were much higher than those in the indoor habitat (S = 71 species, P_m = 15.3%, MA = 2.8 mites/rat, MI = 18.4 mites/rat). In comparison with the closed and simple indoor habitats with relatively low humidity, the outdoor habitats in Yunnan are much more complicated and the humidity in the opened outdoor habitats is generally higher than that in the closed indoor habitats. Most chigger mites prefer to choose hot and humid environments for their development and reproduction [39, 40], and this may partly explain why infestations of chigger mites on *R. tanezumi* are higher in the outdoor habitats than in the indoor habitats. In addition, there are much more species of animal hosts (rodents and some other small mammals) in the outdoor habitats than in the indoor habitats and this allows the outdoor rats to harbor more species and individuals of the mites via cross-infestation because most chigger mites have low host specificity [7,21].

The infestation fluctuation of chigger mites on *R. tanezumi* also occurred along different altitude gradients. The species similarity of chigger mites was very low along different altitude gradients with less than 0.31 of Jaccard indexes ($C_J < 0.31$). The overall infestation prevalence (P_m) and overall mean abundance (*MA*) were highest at the lowest altitude gradient (< 500 m). Between the altitude "500-999 m" and "> 2,499 m", the species richness (species diversity) of the chigger mite community on *R. tanezumi* showed a gradually decreased tendency with the increase of altitude gradients. The curves of Cody index and Jaccard index showed a parabolic tendency along the altitude gradients. It is believed that most chigger mites prefer to choose hot and humid environments for their development and reproduction [1], and this may explain why the species diversity and infestations of chigger mites on *R. tanezumi* in the lower altitude gradients (< 1,000 m) are higher than those in the higher altitudes.

The species diversity, species composition and infestations of chigger mites on the same rat species, *R. tanezumi*, varied along different environmental gradients (different geographical regions, habitats and altitudes) and this reflects the heterogeneity of chigger mite community [11,41]. The heterogeneity of chigger mite community suggests that the same species of host can harbor different species of chigger mites with different infestations in different environments. The heterogeneity of chigger mite community is probably associated with the low host specificity of most chigger mites [7,21]. Because of the heterogeneity of chigger mite community on the same species of host along different environmental gradients, the extensive geographical regions with large host samples are recommended in the field investigations, so as to fully and thoroughly reveal the species diversity (species richness), infestations and community characteristics of chigger mites on a certain species of host.

Species abundance distribution and total species estimation of chigger mites

Species abundance distribution is an important issue in community ecology and it is to describe the relationship between the species and individuals in a certain community, that is, the abundance distribution of different species in the community. The species abundance distribution can directly reflect the proportion structure of the dominant, common and rare species in a community [21,42]. Although a number of models had been previously used in the study of species abundance distribution [42,43], it was Fisher and Preston who applied the log-series distribution model and the log-normal distribution model to describe insect communities and bird communities for the first time [27,44]. In the present study, the log-normal distribution model based on Preston' method was used to describe the species abundance distribution of the chigger mite community on *R. tanezumi*. The results showed that the species abundance distribution of the chigger mite community on *R. tanezumi* was successfully fitted by the log-normal distribution model with a high determination coefficient ($R^2 = 0.93$). The expected species of chigger mites showed a descending tendency with the increase of chigger mite individuals and it reveals the fact that the majority of the mite species are very rare with few individuals and few mite species are dominant with abundant individuals. Some rare species of chigger mites on *R. tanezumi* may be cross-infested from some other hosts of rodents and small mammals due to their low host specificity [21,28].

It is impossible to collect all the species in the field investigations because that some rare species are too rare to be found [28,30,38]. Sometimes it is needed to estimate the expected total species within a given community in ecological studies [30,45]. There are a lot of methods to estimate the total species and the present study used 2 types of estimation methods, Chao 1 formula and the method based on Preston's lognormal model (simply Preston's method) [27,29]. Based on Chao 1 formula, the expected total species of chigger mites on *R. tanezumi* was estimated to be 199 species ($S^* = 199$) with 68 species probably missed in the actual field investigation. Based on Preston's method, the expected total species of chigger mites on *R. tanezumi* was estimated to be 234 species ($S_T = 234$) with 103 species probably missed in the investigation. Although the expected total species of chigger mites estimated by 2 types of methods were different, the estimated total mite species (199 species or 234 species) were much beyond the actual collected 131 species and this indicated that lots of rare mite species had been missed in the investigation. The results imply that the Asian house rat (*R. tanezumi*) has a great potential to harbor abundant species of chigger mites, and the species diversity and composition of chigger mites on the same species of host (*R. tanezumi*) vary along different environmental gradients. To fully and thoroughly reveal the species diversity, infestations and community characteristics of chigger mites on a certain species of rodent host, a wide geographical region with a large host sample are needed in the field investigations. The investigations from a narrow and limited geographical scope without enough host samples would miss lots of mite species and even lead to the misunderstanding of the real status. In the present study, 131 species of chigger mites were found on *Rattus tanezumi*, of which only 1 individual was collected for 37 rare species. The result reflects the fact that some rare species are extremely rare in a certain community.

Although there are a variety of methods to estimate the expected total number of species in a certain community in ecological studies, it is still very difficult to accurately estimate the total species of the ectoparasite communities, including the chigger mite community. In the present study, the estimated total mite species may not be very accurate, but some species were definitely missed in the actual investigation. The expected total species estimated by different types of estimation methods are often different. Chao 1 formula (S^*) used in the present study is based on 2 kinds of rare species with 1 or 2 individuals and it is often used to estimate the expected total species of the communities with lots of rare species [29]. The species abundance distribution of the chigger mite community on *R. tanezumi* showed that the majority of chigger mite species were rare species with few individuals collected (Table 7; Fig. 4). In this situation, it is probably more suitable to use Chao 1 formula (S^*) to estimate the expected total mite species. Thereotically, if the majority of species are common species in a community, it is often recommendable to adopt the estimation method based on Preston's lognormal model (Preston's method) to estimate the expected total species [28,30]. In the present study, the majority of chigger mite species were rare species, not common and dominant species. The expected total mite species estimated by Preston's method may have a relatively high deviation. In this study, however, we are unable to determine which estimation method is the best way to estimate the total number of mite species, and more researches may be needed.

Species-plot relationship

The species-plot relationship is to illustrate the relationship between the sampling plots (the individuals of *R. tanezumi* in this paper) and the number of species within a certain community [25,26,46]. For an "even or uniform" community without much heterogeneity, theoretically the number of species would rapidly increase with the increase of the sampling plots at the beginning of a sampling investigation. When the sampling plots are quite enough, the increasing speed of species would gradually become slower and slower, and even stop increasing eventually. When the number of species stops increasing, a "platform stage" would occur on the curve of species-plot relationship [26,46]. In the present study, however, the curve tendency of the species-plot relationship showed that the number of chigger mite species still kept "going-up" when the sampling plots (individuals of *R. tanezumi*) reached 2,761 individual rats, a large host sample. The result suggests that the chigger mite community on *R. tanezumi* is not an even or uniform community. The species diversity, species composition and infestations on the same species of host (*R. tanezumi*) vary along different environmental gradients with an obvious heterogeneity. Although the sampling plots (individuals of *R. tanezumi*) reached 2,761 individual rats (a large host sample), the number of chigger mite species still kept increasing without the appearance of an expected "platform stage" on the curve of the species-plot relationship. In ecological applications, by the illustration of the species-plot relationship, the minimum sampling size for an even (uniform) community could be roughly estimated [25,47]. The chigger mite community on *R. tanezumi* in Yunnan, however, was an uneven community with very high heterogeneity. In this situation, it is very difficult to estimate the minimum sampling size of the rat hosts because there is no expected "platform stage" appearing on the species-plot curve. Theoretically, if a field investigation infinitely continues, more and more rare species of chigger mites would be found and an expected "platform stage" would probably appear on the species-plot curve. In practice, however, it seems impossible to conduct an infinite investigation. The result of the present study further suggests that it is not suitable to estimate the minimum sampling size of animal hosts according to the depiction of species-plot curve when the studied communities are uneven communities with very high heterogeneity.

In conclusion, the Asian house rat (*R. tanezumi*) is an important infectious source and reservoir host of many zoonoses including scrub typhus, and chigger mites are the exclusive vector of scrub typhus. Yunnan Province is an important focus of scrub typhus in China. It is of significance to study the chigger mites on *R. tanezumi* in Yunnan. The rat *R. tanezumi* in Yunnan has a great potential to harbor abundant species of chigger mites. *Leptotrombidium deliense*, the most powerful vector of scrub typhus in China, is the first major dominant species of chigger mites on *R. tanezumi*, and this greatly increases the risk of transmitting scrub typhus from the rats to humans.

The chigger mite community on *R. tanezumi* in Yunnan is an uneven community with very high heterogeneity. Wide geographical regions with large host samples are recommended in the investigations of chigger mites.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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