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Analysis on infestation and related ecology of chigger mites on large Chinese voles (*Eothenomys miletus*) in five provincial regions of Southwest China

Bei Li[a], Xian-Guo Guo[a,*], Tian-Guang Ren[b], Pei-Ying Peng[c], Wen-Yu Song[a], Yan Lv[a], Peng-Wu Yin[a], Zhe Liu[a,d], Xin-Hang Liu[a,e], Ti-Jun Qian[a]

[a] *Institute of Pathogens and Vectors, Yunnan Provincial Key Laboratory for Zoonosis Control and Prevention, Dali University, Dali, Yunnan, 671000, China*
[b] *Nursing College of Dali University, Dali, Yunnan, 671000, China*
[c] *Institute of Microbiology, Qujing Medical College, Qujing, Yunnan, 655011, China*
[d] *Beijing Health Vocational College, Beijing, 102402, China*
[e] *Central Hospital of Yingkou Economic and Technological Development Zone, Yingkou, Liaoning, 115007, China*

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ABSTRACT

Based on a long-term field investigation in the five provincial regions of Southwest China between 2001 and 2019, the present paper studied the infestation and related ecology of chigger mites (chiggers) on the large Chinese vole (*Eothenomys miletus*), an endemic and dominant rodent species in the regions. A total of 52331 chiggers were collected from 2661 voles, and 52261 mites were identified as 185 species and 13 genera in the family Trombiculidae with very high species diversity. The identified 185 chigger species on *E. miletus* (a single rodent species) even exceeded those recorded in some countries. The overall infestation prevalence (P_m = 53.96%), mean abundance (MA = 19.64) and mean intensity (MI = 36.39) on *E. miletus* were much higher than those on some other rodent species in the same regions. Although the species composition showed a moderate similarity (J = 0.63) between male and female hosts (*E. miletus*), the infestation indices (P_m = 56.25%, MA = 21.67) of chiggers on male hosts were higher than those on the females (P_m = 51.23%, MA = 17.09) ($P < 0.05$). Two dominant chigger species, *Leptotrombidium scutellare* (C_r = 19.17%) and *L. sinicum* (C_r = 11.06%), showed an aggregated distribution pattern among different individuals of their host *E. miletus*, and a relatively high degree of positive association existed between the two dominant chigger species with PCC = 0.57, DI = 0.60 and OI = 0.62 (x^2 = 857.46, $P < 0.001$). *Leptotrombidium densipunctatum*, *Walchia koi*, *Helenicula hsui*, *L. scutellare* and *W. ewingi* showed a high degree of environmental adaptability to their environments with high niche breadths. The theoretical curve of the species abundance distribution of chigger community on *E. miletus* was successfully fitted with Preston's lognormal distribution model. Based on the theoretical curve fitting, the expected total number of chigger species on *E. miletus* was roughly estimated to be 223 species, and 38 chigger species were probably missed in the sampling investigation.

1. Introduction

Chigger mites (trombiculid mites) are a large group of tiny arthropods and they are widely distributed in the world (Shatrov and Kudryashova 2006; Nielsen et al., 2021). It has been controversial about the taxonomic status of chigger mites. In some literature, all chigger mites have been grouped into one family (Trombiculidae) under the order Trombidiformes (Li et al., 1997; Stekolnikov 2021), which is followed by the present study. In some other literature, however, chigger mites have been placed in two families (Trombiculidae and Leeuwenhoekiidae) under the order Trombidiformes (Vercammen--Grandjean and Langston 1976; Nielsen et al., 2021). Of the complex life cycle of chigger mites, only the larval stage (often called "chiggers") is the ectoparasite of other animals (the hosts), especially rodents and other small mammals (Li et al., 1997; Shatrov and Kudryashova 2006; Chaisiri et al., 2019). Chiggers are the exclusive vector of *Orientia tsutsugamushi*, the causative agent of scrub typhus (tsutsugamushi disease). Scrub typhus is an acute febrile zoonosis (zoonotic disease), which is

* Corresponding author.
E-mail address: xgguo2002@163.com (X.-G. Guo).

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prevalent in some parts of Asian (including China) and pacific regions with about one million new cases each year and more than one billion people at risk (Chakraborty and Sarma 2017). From 2006 to 2017, the annual incidence of scrub typhus increased more than 21 times in Southwest China (Xin et al., 2020). Besides transmitting *O. tsutsugamushi*, some chiggers are also suspected to be the potential vector of hantavirus, the pathogen of hemorrhagic fever with renal syndrome (HRFS) (Yu and Tesh 2014; Ding et al., 2021; Xiang et al., 2021). Accounting for 24.5% of China 's land area, Southwest China is a huge geographical territory covering five provincial regions, Yunnan, Sichuan, Guizhou, Chongqing and Tibet (Xizang Autonomous Region), and it is an important focus of scrub typhus and HFRS in China (Zhang et al., 2011a).

Belonging to the genus *Eothenomys* and the family Cricetidae in the order Rodentia, the large Chinese vole or Yunnan red-backed vole (*Eothenomys miletus* Thomas, 1914) is an endemic rodent species in China, and it is mainly distributed in Southwest China (Huang et al., 1995; Zhang 2011; Jiang et al., 2016; Wei et al., 2021). As a dominant rodent species in the distributed regions, *E. miletus* often destroys crops and plants as an important pest in agriculture and forestry. Besides, *E. miletus* is also an important infectious source and reservoir host of some zoonoses such as plague, endemic typhus (murine typhus), scrub typhus and HRFS, etc. (Zheng et al., 2007; Zhang et al., 2011b; Guo et al., 2013).

Based on field investigations in three provincial regions (Yunnan, Guizhou and Sichuan) of Southwest China between 2001 and 2013, a previous study in our research group once reported chigger mites on *E. miletus* in the investigated regions (Peng et al., 2016b). The field investigations in the previous study, however, did not cover all the five provincial regions of Southwest China because of financial limitation at the time (Li et al., 2021). To continue and deepen the previous study, field investigations between 2001 and 2019 in the present study covered all the five provincial regions of Southwest China and investigation sites increased from previous 39 sites to the present 91 sites. The present study is an attempt to illustrate the species composition and diversity, overall infestation and the related ecological issues of chiggers on *E. miletus* in the whole Southwest China, which will update the knowledge of chiggers on *E. miletus* and provide more detail and comprehensive information for the surveillance of chiggers and some other related studies in the region.

2. Materials and methods

2.1. Field investigation and collection of chigger mites

The field investigations were conducted in 91 investigation sites between 2001 and 2019, which covered all the five provincial regions of Southwest China, Yunnan, Sichuan, Guizhou, Chongqing and Tibet (Fig. 1, Appendix). The investigations in 39 of the total 91 sites had been made in three provincial regions of Southwest China (Yunnan, Guizhou and the South of Sichuan) between 2001 and 2013 in the previous studies (Peng et al., 2016b). The rest 52 sites were newly added in other two provincial regions, Chongqing and Tibet after 2013 (Fig. 1, Appendix). Of the total 91 sites, only three were in eastern Tibet, and no investigation was conducted in western Tibet, a vast plateau where *E. miletus* has not been recorded so far (Huang et al., 2008). In each investigation site, rodents (rats, mice and voles) and other small mammals (insectivores and tree shrews) were captured with mouse traps (18 × 12 × 9 cm, Guixi Mousetrap Apparatus Factory, Guixi, Jiangxi, China), which were randomly placed in different habitats (residential area, farmland, bush and woodland) in the evening and then checked the next morning (Peng et al., 2015, 2016b). Each trapped small mammal (animal host) was separately placed in a white cloth bag and then transported to the field laboratory where ectoparasitic chiggers were conventionally collected, and the collected chiggers were preserved in a vial containing 70% ethanol (Kennedy 1976; Li et al., 1997). After the collection of chiggers, each animal host was identified into species according to its appearance (body size, shape and hair color), body measurements (body weight, body length, tail length, ear height and hind foot length) and other morphological characteristics (Huang et al., 1995; Wlison et al., 2017). In the laboratory, the collected chiggers were mounted onto glass slides with Hoyer' s solution. After dehydration, drying and transparency, the mounted chigger specimens were identified into species under microscopes (Kennedy 1976; Li et al., 1997; Stekolnikov 2013). Based on the identification of animal hosts and chiggers, all the large Chinese voles (*E. miletus*), together with the

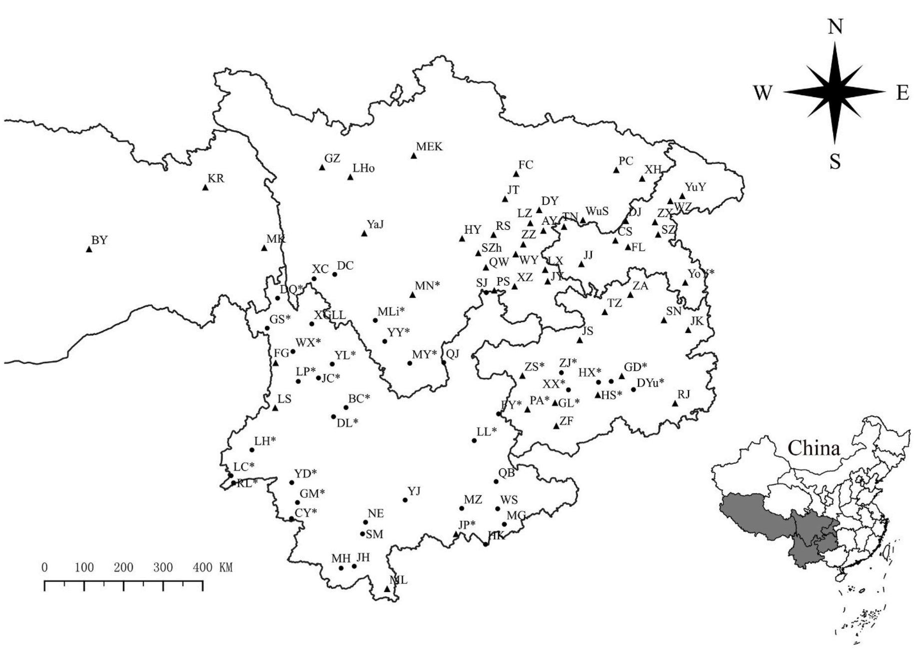

Fig. 1. Investigation sites (n = 91) in the five provincial regions of Southwest China between 2001 and 2019 (The sites marked "▲" were newly increased sites after 2013 and those marked "*" were the sites where large Chinese voles, *E. miletus*, were captured. The name abbreviations of the investigation sites were shown in "Appendix").

chiggers on the body surface of the voles, were chosen as the target of the present study. The capture of animal hosts was officially permitted by the local authority of wildlife service. The use of animals for research was officially approved by Animals' Ethics Committee of Dali University. Representative specimens of animal hosts and chiggers with clear and typical morphological characteristics were deposited in the specimen repository of Institute of Pathogens and Vectors, Dali University, Dali, Yunnan, China.

2.2. Statistical analysis on infestation of E. miletus with chiggers

According to the conventional statistical methods, the constituent ratio (C_r) was used to calculate the proportion of each chigger species on *E. miletus*. The prevalence (P_m), mean abundance (*MA*) and mean intensity (*MI*) were used to analyze the infestation of *E. miletus* with chiggers. Jaccard' s similarity index (*J*) was used to compare the species similarity of chiggers between male and female hosts (Margolis et al., 1982; Liu et al., 2019; Xiang et al., 2021; Li et al., 2022).

2.3. Analysis on spatial distribution pattern and interspecific relationship of dominant chigger species

The patchiness index (m^*/m), clump index (*I*) and Cassie index (C_A) were used to analyze the spatial distribution pattern of dominant chigger species on *E. miletus* (Lloyd 1967; Costa et al., 2010; Liu et al., 2019).

Based on a 2 × 2 contingency table (see Table 4 in "Results"), Chi-square test (x^2) was used to qualitatively determine the interspecific association between any two dominant chigger species (Xu et al., 2016), and then Pearson correlation coefficient (*PCC*), Dice coefficient (*DI*) and Ochiai coefficient (*OI*) were used to quantitatively measure the degree of the interspecific association (Yule 1912; Driver and Kroeber 1932; Xu et al., 2016).

$$x^2 = \frac{(ad-bc)^2 n}{(a+b)(c+d)(a+c)(b+d)};\ PCC = \frac{ad-bc}{\sqrt{(a+b)(a+c)(b+d)(c+d)}};\ DI = \frac{2a}{2a+b+c};\ OI = \frac{a}{\sqrt{a+b}\sqrt{a+c}}$$

In the above formulae, the specific meaning of *a*, *b*, *c*, *d* and *n* were shown in the annotation of Table 4 in "Results". In the analysis of Chi-square test (x^2), a positive association is determined to exist between two chigger species when $ad > bc$, while a negative association between two chigger species when $ad < bc$. The value of *PCC* ranges from −1 to 1. The closer to 1 the *PCC* value is, the stronger the positive association would be. The closer to −1 the *PCC* value is, the stronger the negative association would be. When the *PCC* value reaches 0, the two chigger species would be considered to be mutually independent. The values of *DI* and *OI* range from 0 to 1. The closer to 1 the *DI* and *OI* values are, the stronger the positive association would be.

2.4. Analysis on niche breath and overlap of main chigger species

Based on the constituent ratios (C_r) of some main species of chiggers on *E. miletus* in different environment gradients or environment series (different altitudes, latitudes and habitats), Shannon-Wiener's niche breadth (B_i) was used to measure the environmental niche breadth of a certain chigger species in a certain environment series (Colwell and Futuyma 1971; Peng et al., 2017). According to B_i calculation, the multidimensional niche breadth (B_{in}) based on the principal component analysis (PCA) was used to evaluate the comprehensive niche breadth of a certain chigger species in the multidimensional environment series, the combined environment series of altitudes, latitudes and habitats (Xu 1999; Shi et al., 2006). The cosine similarity ($\cos\theta_{ij}$) was used to evaluate the environmental niche overlaps of different chigger species (species *i* and *j*) in the selection of different environment series, altitudes, latitudes and habitats (Guo 1999; Luo and Guo 2015). The above niche overlaps were expressed in the dendrogram of hierarchical clustering analysis which was done under SPSS 20 (Luo and Guo 2015).

$$B_i = \frac{\lg\sum N_{ij} - \left(1/\sum N_{ij}\right)\left(\sum N_{ij}\lg N_{ij}\right)}{\lg r};\ B_{in} = \sqrt{\sum_{i=1}^{n} B_i^{\,2}};\ \cos\theta_{ij} = \frac{\sum_{h=1}^{n} P_{ih}\cdot P_{jh}}{\sqrt{\sum_{h=1}^{n} P_{ih}^2 \cdot \sum_{h=1}^{n} P_{jh}^2}}$$

In the above formulae, N_{ij} = the number of chigger species *i* on grade *j* of a certain environment series (altitudes, latitudes or habitat in the present paper), *r* = the number of grades in a certain environment series, *n* = the number of grades in the combined environment series, P_{ih} = the constituent ratio of chigger species *i* on grade *h* of a certain environment series, and P_{jh} = the constituent ratio of chigger species *j* on grade *h* of a certain environment series. The values of B_i, B_{in} and $\cos\theta_{ij}$ range from 0 to 1. When the values of B_i and B_{in} are close to 0, it means that there is nearly no distribution of the examined chigger species in a certain environment series. When the values of B_i and B_{in} reach 1, it means that the examined chigger species is distributed in all the grades of a certain environment series.

2.5. Description of species abundance distribution

In the present study, all the chigger species on *E. melitus* were considered a community unit. The species abundance distribution of the chigger community was depicted in a semi-logarithmic coordinate system, in which the X-axis representing chigger individuals was labeled with log intervals based on $\log_3$N and the Y-axis standing for chigger species was labeled with arithmetic scales. The following lognormal distribution model based on Preston's method was used to fit the theoretical curve of the species abundance distribution, and the determination coefficient (R^2) was used to evaluate the fitting goodness of the theoretical curve (Preston 1948; Peng et al., 2017; Liu et al., 2019; Ding et al., 2021).

$$\widehat{S}(R) = S_0 e^{-[\alpha(R-R_0)]^2} \text{ (The lognormal distribution model)}$$

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

In the above formulae, $\widehat{S}(R)$ = the theoretical number of chigger species in the *R*-th log interval, R_0 = the corresponding log interval at the highest point of the actual curve of species abundance, S_0 = the number of chigger species at R_0 log interval, e = 2.7182… (the base number of natural logarithm), α = the spread constant which is determined according to the highest determination coefficient (R^2) in the fitting process of the theoretical curve, $S(R)$ = the actual number of chigger species in *R*-th log interval, *m* = the number of log intervals and $\bar{S}(R)$ = the average number of chigger species at each log interval.

2.6. Estimation of expected total species

According to the fitting result of the theoretical curve of species abundance, the expected total number of chigger species on *E. miletus* (S_T), together with the number of probably missed chigger species in the sampling investigation (S_M), were roughly estimated with the following formulae (Ding et al., 2021; Chen et al., 2022).

$$S_T = \left(S_0\sqrt{\pi}\right)/\alpha;\ S_M = S_T - S_A$$

Table 1
Identified chigger mites (chiggers) from large Chinese voles (*E. miletus*) in the five provincial regions of Southwest China (2001–2019).

Names of chigger mites	Individuals	Names of chigger mites	Individuals
Leptotrombidium scutellare (Nagayo et al., 1921)	10019	*T. bambusoides* Wang et Yu, 1965	489
L. sinicum Yu et al., 1981	5778	*T. qianye* Wen et al., 1984	32
L. eothenomydis Yu et Yang, 1986	3929	*T. chilie* Wen et Xiang, 1984	27
L. densipunctatum Yu et al., 1982	3336	*T. nujiange* Wen et Xiang, 1984	17
L. hiemalis Yu et al., 1982	3066	*T. alpinus* Yu et Yang, 1979	3
L. rusticum Yu et al., 1986	*1804*	*T. kuanye* Wen et Xiang, 1984	2
L. bambicola Wen et Xiang, 1984	986	*T. shaoye* Wen et al., 1984	2
L. jinmai Wen et Xiang, 1984	698	*Neotrombicula microti* (Ewing, 1928)	194
L. wangi Yu et al., 1986	634	*N. microtomici* Wen et al., 1984	72
L. gongshanense Yu et al., 1981	555	*N. deqinensis* Yu et Wang, 1981	27
L. ejingshanense Yu et al., 1982	446	*N. aeretes* Hus et Yang, 1985	8
L. yui (Chen et Hsu, 1955)	389	*N. marmotae* Wen et al., 1984	6
L. bishanense Yu et al., 1986	379	*N. japonica* (Tanaka et al., 1930)	5
L. baoshui Wen et Xiang, 1984	265	*N. tongtianhensis* Yang et al., 1995	4
L. deliense (Walch, 1922)	228	*N. sinica* (Wang, 1964)	3
L. biji Wen and Xiang, 1984	223	*N. longmenis* Wen et Xiang, 1984	2
L. yongshengense Yu et Yang, 1986	187	*N. gamaensis* Yang, 1985	1
L. dianchi Wen et Xiang, 1984	128	*Helenicula simena* (Hsu et Chen, 1957)	4398
L. yunlingense Yu et Zhang, 1981	119	*H. hsui* Zhao, 1990	746
L. laojunshanense Yu et al., 1986	116	*H. yunnanensis* Wen et Xiang, 1984	38
L. robustisetum Yu et al., 1983	105	*H. miyagawai* (Sasa kunada et Miura, 1951)	24
L. caudatum Wen et al., 1984	105	*H. abaensis* Wang et al., 1984	23
L. rupestre Traub et Nadchatram, 1967	104	*H. globularis* (Walch, 1927)	14
L. shuqui Wen et Xiang, 1984	70	*H. kohlsi* (Philip et Woodward, 1964)	13
L. longchuanense Yu et al., 1981	67	*H. saihsuensis* Hsu et Chen, 1964	10
L. hsui Yu et al., 1986	57	*H. edibakeri* Nadchatram et Traub, 1971	7
L. suense Wen, 1984	57	*H. olsufjevi* (Schulger, 1955)	4
L. alpinum Yu et Yang, 1986	54	*H. rattihaikonga* (Hsu et Chen, 1957)	1
L. dihumerale Traub et Nadchatram, 1967	53	*H. aulacochaeta* Sun et al., 1986	1
L. akamushi Barumpt, 1910	53	*Herpetacarus hastoclavus* Yu et al., 1979	3160
L. allosetum Wang et al., 1981	50	*Herpetacarus tenuiclavus* Yu et al., 1979	205
L. yunnanense Yu et al., 1980	50	*Herpetacarus tengchongensis* Yu et al., 1980	7
L. xiaowei Wen et Xiang, 1984	48	*Herpetacarus limon* (Wen et Xiang, 1984)	5
L. sexsetum Yu et Hu, 1981	47	*Herpetacarus bisetus* Yu et Duan, 1980	3
L. muntiaci Wen et Xiang, 1984	47	*Walchiella notiala* Yu et al., 1981	1
L. imphalum Vercammen-Grandjean et Langston, 1975	46	*Ascoschoengastia leechi* (Domrow, 1962)	31
L. rufocanum Wang et Liu, 1989	46	*A. sifanga* Wen et al., 1984	2
L. xiaguanense Yu et al., 1981	42	*A. indica* (Hirst, 1915)	1
L. kitasatoi (Fukuzum et Obata, 1956)	35	*Schoengastia cantomensis* Liang et al., 1957	1
L. dongluoense Wang et al., 1981	32	*Walchia koi* (Chen et Hsu, 1957)	1425
L. spicanisetum Yu et al., 1986	26	*W. ewingi* (Fuller, 1949)	763
L. bayanense Yang, 1994	25	*W. zangnanica* Wu et Wen, 1984	81
L. kaohuense Yang et al., 1959	22	*W. chuanica* Wen et Song, 1984	77
L. lianghense Yu et al., 1983	20	*W. enode* Gater, 1932	59
L. longimedium Wen et Xiang, 1984	20	*W. micropelta* (Traub et Evans, 1957)	52
L. apodevrieri Wen et Xiang, 1984	20	*W. xishaensis* Zhao et al., 1986	38
L. sheshui Wen et Xiang, 1984	16	*W. chinensis* (Chen et Hsu, 1955)	19
L. qujingense Yu et al., 1981	14	*W. parapacifica* (Chen et al., 1955)	35
L. apodemi Wen et Sun, 1984	13	*W. turmalis* (Gater, 1932)	17
L. fujianense Liao et Wang, 1983	13	*W. acutascuta* Chen, 1980	13
L. cangjiangense Yu et al., 1981	12	*W. kritochaeta* (Traub et Evans, 1957)	8
L. qiui Yu et al., 1986	11	*W. shui* Wen et Song, 1984	4
L. zhongdianense Yu et Yang, 1981	11	*W. szechuanica* (Teng, 1963)	3
L. biluoxueshanense Yu et al., 1982	11	*W. nanfangis* Wen et Xiang, 1984	1
L. huangchuanense Yang, 1994	11	*W. cordiopelta* Wen et Xiang, 1984	1
L. parapalpale (Womersley, 1952)	10	*W. rustica* (Gater, 1932)	1
L. trapezoidum Wang et al., 1981	9	*Intermedialia hegu* (Yu, Yang et Wu, 1979)	47
*L. linhuaikongense (*Wen et Hsu, 1961)	9	*I. bingbi* Wen et Xiang, 1984	3
L. rubellum Wang et Liao, 1984	7	*I. guangxiensis* Zhou et Wen, 1984	1
L. filasensillum Wang et Song, 1982	7	*I. xuedun* Wen et Xiang, 1984	1
L. linji Wen et Sun, 1984	7	*I. xidun* Wen et Xiang, 1984	1
L. cuonae Wang et al., 1996	6	*Gahrliepia longipedalis* Yu et Yang, 1986	1182
L. bawangense Zhao, 1982	6	*G. linguipelta* Jeu et al., 1983	938
L. rectanguloscutum (Hsu et Chen, 1964)	6	*G. yunnanensis* Hsu et al., 1965	496
L. saltuosum Yu et al., 1982	5	*G. radiopunctata* Hsu et al., 1965	327
L. shuyui Wen et al., 1984	5	*G. silvatica* Yu et Yang, 1982	286
L. deplanoscutum Yu et Zi, 1981	4	*G. deqinensis*Yu et Yang, 1982	286
L. lushanense Wang et Song, 1991	4	*G. orientalis* Wen et Xiang, 1984	102
L. nyctali Wen et Sun, 1984	4	*G. chekiangensis* Chu, 1964	89
L. pallidum (Nagayo et al., 1919)	3	*G. latiscutata* Chen et Fan, 1981	75
L. intermedium Nagayo et al., 1920	3	*G. miyi* Wen et Song, 1984	53
L. gemiticulum (Traub et al., 1958)	3	*G. xiaowoi* Wen et Xiang, 1984	17
L. yulini Wen et Xiang, 1984	3	*G. lamella* Chen et al., 1980	17
L. quadrifurcatum Wen et Xiang, 1984	2	*G. eurypunctata* Jeu et al., 1983	15

(*continued on next page*)

Table 1 (*continued*)

Names of chigger mites	Individuals	Names of chigger mites	Individuals
L. jianshanense Yu et al., 1982	2	*G. agrariusia* Hus et al., 1965	11
L. liaoji Wen et Sun, 1984	2	*G. zhongwoi* Wen et Xiang, 1984	10
L. shanghaense Wen and Lu, 1984	2	*G. zayuensis* Wu et Wen, 1984	8
L. heiense Wen, 1984	2	*G. lengshui* Wen et Xiang, 1984	8
L. sixinum Wen et al., 1984	2	*G. meridionalis* Yu et al., 1980	7
L. chuanxi Wen et al., 1984	1	*G. madun* Wen *et* Xiang, 1984	5
L. nudisensillum Yu et al., 1981	1	*G. banyei* Wen et Xiang, 1984	3
*L. sinotupaium*Wen et Xiang, 1984	1	*G. megascuta* Hus et al., 1965	3
L. kawamurai (Fukuzumi et Obata, 1953)	1	*G. octosetosa* Chen et al., 1956	3
L. hupeicum Ma et Hsu, 1965	1	*G. shanyangensis* Huang, 1988	3
L. xishani Wen et Xiang, 1984	1	*G. pintanensis* Wang, 1962	2
L. guzhangense Wang et al., 1985	1	*G. chungkingensis* Jeu et al., 1963	1
L. taishanicum Meng et al., 1983	1	*G. yangchenensis* Chen et Hsu, 1957	1
L. neotebraci Xiang et Wen, 1986	1	*Schoengastiella ligula* Radford, 1946	4
*L. postfoliatum*Wang et al., 1981	1	*Chatia maoyi* Wen et Xiang, 1984	55
L. sialkotense Vercammen-Grandjean et Langston, 1976	1	*C. alpina* Shao et Wen, 1984	2
L. laxoscutum Teng, 1981	1	*C. huanglungensis* (Chang et Wen, 1965)	1
L. myotis (Ewing, 1929)	1	*C. acrichela* Wen et al., 1984	1
Trombiculindus yunnanus Wang et Yu, 1965	1187		

In the above formulae, S_A = the number of actually collected chigger species, $\pi = 3.1415...$ (the circumferential rate), and S_0 and α are the same as before.

3. Results

3.1. Infestation of *E. miletus* with chiggers

A total of 2661 large Chinese voles (*E. miletus*) were captured in 32 of 91 sampled sites (Fig. 1, Appendix), and 52331 chiggers were collected from *E. miletus*. And 52261 of 52331 collected chiggers were identified as 185 species and 13 genera in the family Trombiculidae (Table 1). The remaining 70 chiggers were unidentified because of the absence of key characters (broken body), key characters not clear due to debris, or suspected new species. The unidentified 70 chiggers were not included in the statistical calculation of the present study. The overall prevalence (P_m), mean abundance (*MA*) and mean intensity (*MI*) of *E. miletus* (host) with chiggers were 53.96%, 19.64 chiggers/per host and 36.39 chiggers/per host respectively. The prevalence and mean abundance of chiggers on male hosts ($P_m = 56.25\%$, $MA = 21.67$) were higher than those on female hosts ($P_m = 51.23\%$, $MA = 17.09$) ($P < 0.05$). The mean intensity of chiggers on male hosts ($MI = 38.52$) was also higher than that on female hosts ($MI = 33.37$), but without statistical significance ($P > 0.05$) (Table 2). The species similarity of chiggers was moderately similar between different sexes of the hosts, *E. miletus* ($J = 0.63$).

3.2. Spatial distribution pattern and interspecific association of dominant chigger species on *E. miletus*

Leptotrombidium scutellare ($C_r = 19.17\%$) and *L. sinicum* ($C_r = 11.06\%$) were two dominant chigger species on *E. miletus*, which accounted for 30.23% ($C_r = 30.23\%$) of all the identified 185 chigger species. The patchiness index (m^*/m), clump index (I) and Cassie index (C_A) of two dominant chigger species were showed in Table 3.

According to the 2 × 2 contingency table (Table 4), the value of $a \times d$ ($ad = 473000$) was much higher than that of $b \times c$ ($bc = 12614$) with extremely statistical significance ($x^2 = 857.46$, $P < 0.001$). Pearson correlation coefficient ($PCC = 0.57$), Dice coefficient ($DI = 0.60$) and Ochiai coefficient ($OI = 0.62$) were positive values and they are higher than 0.50.

3.3. Environmental niche breadths and niche overlaps of 18 main chigger species on *E. miletus*

Of the identified 185 chigger species, 18 main species accounted for 85.35% (44604/52261) of all the mites (Table 5, Table 6), and *Leptotrombidium densipunctatum* showed the highest niche breadths along different altitudes ($B_{i1} = 0.66$), latitudes ($B_{i2} = 0.69$), habitats ($B_{i3} = 0.85$) and the combined environment series (multidimensional environment series, $B_{in} = 1.28$) (Table 5). The niche breadths of *Walchia koi* ($B_{in} = 0.95$), and *Helenicula hsui* ($B_{in} = 0.94$), *L. scutellare* ($B_{in} = 0.87$) and *W. ewingi* ($B_{in} = 0.87$) were next to *L. densipunctatum* along the combined environment series (Table 5, Fig. 2). Based on the calculation of the cosine similarity, $\cos\theta_{ij}$ (Table 7), the hierarchical clustering analysis was used to illustrate the comprehensive niche overlaps of the 18 main chigger species along the combined environment series. The dendorgram of the hierarchical clustering analysis showed that the 18 main chigger species were classified into 4 overlapped groups when $\lambda = 4$ (Fig. 3). The first overlapped group included nine chigger species, namely *L. sinicum*, *L. rusticum*, *L. gongshanense*, *L. scutellare*, *Herpetacarus hastoclavus*, *Trombiculindus yunnanus*, *L. jinmai*, *H. simena* and *L. eothenomydis*. There were three chigger species in the second overlapped group (*H. hsui*, *L. wangi* and *L. hiemale*) and five chigger species in the third overlapped group (*Gahrliepia linguipelta*, *W. ewingi*, *W. koi*, *L. densipunctatum* and *G. longipedalis*). *Leptotrombidium bambicola* (only one chigger species), however, formed the fourth branch independently (Fig. 3).

3.4. Species abundance distribution of chigger community on *E. miletus*

The actual curve of species abundance distribution of chigger community on *E. miletus* was depicted in the semi-logarithmic coordinate system. As showed in Table 8 and Fig. 4, the theoretical curve of the species abundance distribution was successfully fitted with Preston's lognormal distribution model with the theoretical equation of $\widehat{S}(R) = 34e^{-[0.27(R-2)]^2}$ ($\alpha = 0.27$, $R^2 = 0.91$).

3.5. Expected total species of chiggers on *E. miletus*

Based on the theoretical curve fitting of species abundance distribution, the expected total number of chigger species on *E. miletus* in the five provincial regions of Southwest China was roughly estimated to be 223 species, and 38 chigger species were probably missed in the sampling investigation.

4. Discussions

4.1. Species composition and infestation of chiggers on *E. miletus*

The result of the present study showed that the identified 185 chigger species on *E. miletus* (a single rodent species) extremely exceeded all the

Table 2
Infestations of chigger mites (chiggers) on different sexes of large Chinese voles (*E. miletus*) in the five provincial regions of Southwest China (2001–2019).

Sexes of the voles	Individuals of the voles		Overall infestations of chiggers on the vole hosts			Constituent ratios (C_r) and species richness (S) of chiggers		
	Examined	Infested	P_m (%)	*MA*	*MI*	Individuals	C_r (%)	*S*
Female	1183	606	51.23	17.09	33.37	20222	38.95	147
Male	1463	823	56.25	21.67	38.52	31701	61.05	167
Total	2646	1429	54.01	19.62	36.34	51923	100.00	185

Annotation: The animal hosts (*E. miletus*) without sex record were not included in the above table.

Table 3
Analysis on spatial distribution pattern of two dominant chigger species on *E. miletus* in the five provincial regions of Southwest China (2001–2019).

Dominant chigger species	Patchiness index (m^*/m)	Clump index (I)	Cassie index (C_A)
Leptotrombidium scutellare	32.52	118.67	31.52
Leptotrombidium sinicum	41.47	87.88	40.47

Table 4
Analysis on interspecific association between dominant chigger species on large Chinese voles (*E. miletus*) in the five provincial regions of Southwest China (2001–2019).

Dominant chigger species		Leptotrombidium *scutellare* (Chigger species Y)		
		+	−	Total
Leptotrombidium *sinicum* (Chigger species X)	+	220 (*a*)	50 (*b*)	273 (*a*+*b*)
	−	238 (*c*)	2150 (*d*)	2388 (*c* + *d*)
Total		458 (*a*+*c*)	2203 (*b* + *d*)	2661 (*n*)
Chi-square		857.46		
Significance		P < 0.001		

Annotation: In the above table, n = the total number of animal hosts (*E. miletus*), a = the host individuals simultaneously infested with chigger species X and Y, b = the host individuals only infested with chigger species X, c = the host individuals only infested with chigger species Y, and d = the host individuals without the infestation of chigger species X and Y.

chigger species previously recorded from multiple species of hosts in a wide region or a whole country in some other countries (Roh et al., 2014; Chaisiri et al., 2016, 2019). The high species diversity of chiggers on *E. miletus* suggests that *E. miletus* has a great potential to harbor abundant chigger species on its body surface (Peng et al., 2016b). Although the field investigation in the present study has covered all the five provincial regions of Southwest China and the investigation sites have increased from previous 39 sites to the present 91 sites, the number of chigger species in the present study (185 species) was not much higher than that (175 species) in the previous study (Peng et al., 2015, 2016b). This suggests that the majority of chigger species has been found on *E. miletus* in Southwest China, and the result of the present study has objectively reflected the real species diversity of chiggers on *E. miletus* in the region. It is unlikely to significantly increase the number of chigger species even if the investigation scope is further expanded because some rare species are too few to be collected (Baltanás 1992; Peng et al., 2016b; Ding et al., 2021).

The overall infestation indices (P_m = 53.96%, *MA* = 19.64, *MI* = 36.39) of chiggers on *E. miletus* in the present study were very close to those on the same host (P_m = 57.69%, *MA* = 20.24, *MI* = 35.08) in the previous report (Peng et al., 2015), and this reflects a relatively stable status of chigger infestation on *E. miletus*. Previously our research group once reported the species composition and infestation of chiggers on two other rodent species, *Apodemus agrarius* and *A. chevrieri*, in Southwest China. The previous reports showed that only 14 chigger species were found on *A. agrarius* with very low infestation indices (P_m = 3.40%, *MA* = 0.36, *MI* = 10.63), and 107 chigger species (higher than on *A. agrarius*) were found on *A. chevrieri* with relatively high infestation indices (P_m = 31.95%, *MA* = 6.32, *MI* = 19.77) (Chen et al., 2021b, 2022). The chigger species diversity and infestation indices on *E. miletus* in the present study were obviously higher than those on *A. agrarius* and *A. chevrieri* in the same region (Chen et al., 2021b, 2022). The species

Table 5
Niche breadths of 18 main chigger species on large Chinese voles (*E. miletus*) in five provincial regions of Southwest China (2001–2019).

Chigger species	Codes	Individuals	Constituent ratios (%)	Niche breaths			
				B_{i1}	B_{i2}	B_{i3}	B_{in}
Leptotrombidium scutellare	1	10019	19.17	0.47	0.28	0.68	0.87
Leptotrombidium sinicum	2	5778	11.06	0.25	0.05	0.48	0.55
Helenicula simena	3	4398	8.42	0.39	0.14	0.55	0.69
Leptotrombidium eothenomydis	4	3929	7.52	0.15	0.39	0.58	0.72
Leptotrombidium densipunctatum	5	3336	6.38	0.66	0.69	0.85	1.28
Herpetacarus hastoclavus	6	3160	6.05	0.25	0.00	0.47	0.53
Leptotrombidium hiemale	7	3066	5.87	0.02	0.49	0.53	0.72
Leptotrombidium rusticum	8	1804	3.45	0.30	0.16	0.56	0.65
Walchia koi	9	1425	2.73	0.51	0.47	0.65	0.95
Trombiculindus yunnanus	10	1187	2.27	0.17	0.01	0.41	0.45
Gahrliepia longipedalis	11	1182	2.26	0.42	0.08	0.32	0.53
Leptotrombidium bambicola	12	986	1.89	0.20	0.20	0.23	0.36
Gahrliepia linguipelta	13	938	1.79	0.53	0.04	0.47	0.71
Walchia ewingi	14	763	1.46	0.50	0.40	0.59	0.87
Helenicula hsui	15	746	1.43	0.03	0.63	0.69	0.94
Leptotrombidium jinmai	16	698	1.34	0.14	0.04	0.64	0.66
Leptotrombidium wangi	17	634	1.21	0.09	0.14	0.73	0.75
Leptotrombidium gongshanense	18	555	1.06	0.46	0.12	0.67	0.82

Annotation: B_{i1} = the niche breaths along different altitudes; B_{i2} = the niche breaths along different latitudes; B_{i3} = the niche breaths along different habitats; B_{in} = the niche breaths along the combined environment series (multidimensional environment series).

Table 6
Constituent ratios (C_r) of the 18 main chigger species on large Chinese voles (*E. miletus*) along different environment series (altitudes, latitudes and habitats) in the five provincial regions of Southwest China (2001–2019).

Codes of chigger species	Constituent ratios (C_r, %) of chigger species at different altitudes (m)				Constituent ratios (C_r, %) of chigger species at different latitudes (°N)				Constituent ratios (C_r, %) of chigger species at different habitats			
	<1000	1000–2000	2000–3000	≥3000	<24	24–26	26–28	≥28	Woodland	Farmland	Bush	Residential area
1	0.00	0.09	0.80	0.11	0.00	0.87	0.13	0.00	0.14	0.59	0.28	0.00
2	0.00	0.08	0.90	0.01	0.00	0.99	0.01	0.00	0.01	0.70	0.29	0.00
3	0.00	0.23	0.77	0.00	0.00	0.96	0.02	0.02	0.01	0.31	0.66	0.03
4	0.00	0.02	0.96	0.02	0.00	0.84	0.08	0.08	0.04	0.37	0.59	0.00
5	0.00	0.38	0.53	0.09	0.00	0.58	0.16	0.27	0.08	0.53	0.19	0.20
6	0.00	0.11	0.89	0.00	0.00	1.00	0.00	0.00	0.00	0.35	0.65	0.00
7	0.00	0.00	1.00	0.00	0.00	0.58	0.42	0.00	0.02	0.36	0.62	0.00
8	0.00	0.13	0.87	0.01	0.00	0.95	0.05	0.00	0.06	0.68	0.26	0.00
9	0.00	0.54	0.45	0.00	0.00	0.74	0.02	0.23	0.01	0.61	0.30	0.07
10	0.00	0.06	0.94	0.00	0.00	1.00	0.00	0.00	0.00	0.24	0.76	0.00
11	0.00	0.78	0.20	0.02	0.00	0.98	0.02	0.00	0.03	0.88	0.09	0.00
12	0.00	0.92	0.07	0.00	0.00	0.07	0.00	0.92	0.00	0.01	0.06	0.92
13	0.00	0.52	0.47	0.01	0.00	0.99	0.01	0.00	0.00	0.67	0.33	0.00
14	0.00	0.45	0.55	0.00	0.00	0.84	0.07	0.09	0.04	0.53	0.43	0.00
15	0.00	0.01	0.99	0.00	0.00	0.62	0.31	0.07	0.46	0.11	0.43	0.00
16	0.00	0.04	0.96	0.01	0.00	0.99	0.01	0.00	0.15	0.21	0.65	0.00
17	0.00	0.00	0.97	0.02	0.00	0.96	0.04	0.01	0.39	0.15	0.45	0.00
18	0.00	0.19	0.78	0.03	0.00	0.96	0.03	0.00	0.11	0.64	0.24	0.01

Annotation: The codes of chigger species are the same as in Table 5.

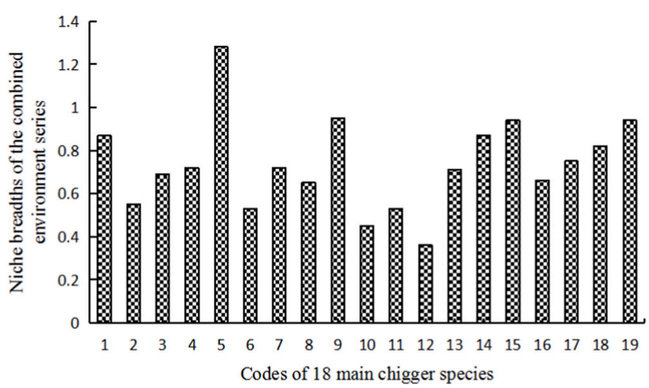

Fig. 2. Niche breaths of the 18 main chigger species on large Chinese voles (*E. miletus*) along the combined environment series (multidimensional environment series) in the five provincial regions of Southwest China (2001–2019).

diversity and infestation of chiggers are often influenced by a series of factors including host species and various environmental factors. Different host species usually have different susceptibility to chiggers and other ectoparasites (Kaplan and Denno 2007; Shenbrot et al., 2007; Yin et al., 2021). The high species diversity and infestation of chiggers on *E. miletus* suggest that *E. miletus* is highly susceptible to chigger infestation. The majority of chiggers usually have a low host specificity, and cross infestations of chiggers often happen among different host species in different environments (Shatrov and Kudryashova 2006; Peng et al., 2016a; Li et al., 2022). *Eothenomys miletus* is often distributed in various wild habitats such as farmlands, bushes and woodlands (Huang et al., 1995; Wlison et al., 2017), and this may allow *E. miletus* to harbor abundant chiggers through cross infestations with other animal hosts (Kaplan and Denno 2007; Mu and Zhu 2015; Chen et al., 2022).

Although the species composition showed a moderate similarity ($J = 0.63$) between male and female *E. miletus*, the infestation indices ($P_m = 56.25\%$, $MA = 21.67$) of chiggers on the male *E. miletus* were higher than those on the females ($P_m = 51.23\%$, $MA = 17.09$) ($P < 0.05$), which revealed the sex-bias of *E. miletus* when infesting with chiggers. The

Table 7
Niche overlaps (cosine similarity, $\cos\theta_{ij}$) of the 18 main chigger species on large Chinese voles (*E. miletus*) along the combined environment series (multidimensional environment series) in the five provincial regions of Southwest China (2001–2019).

Codes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	0.99	1.00																
3	0.93	0.93	1.00															
4	0.95	0.95	0.97	1.00														
5	0.90	0.89	0.84	0.84	1.00													
6	0.94	0.95	0.99	0.99	0.83	1.00												
7	0.90	0.87	0.90	0.95	0.79	0.92	1.00											
8	0.99	1.00	0.93	0.94	0.90	0.94	0.87	1.00										
9	0.88	0.88	0.87	0.82	0.96	0.84	0.73	0.89	1.00									
10	0.91	0.91	0.99	0.98	0.78	0.99	0.92	0.90	0.79	1.00								
11	0.77	0.78	0.72	0.63	0.85	0.68	0.53	0.80	0.93	0.61	1.00							
12	0.11	0.10	0.18	0.11	0.50	0.12	0.07	0.12	0.45	0.10	0.33	1.00						
13	0.91	0.92	0.90	0.84	0.91	0.88	0.74	0.92	0.97	0.83	0.95	0.27	1.00					
14	0.93	0.93	0.95	0.90	0.94	0.93	0.83	0.94	0.98	0.89	0.89	0.30	0.98	1.00				
15	0.86	0.82	0.85	0.91	0.75	0.87	0.92	0.82	0.66	0.88	0.46	0.10	0.67	0.76	1.00			
16	0.92	0.92	0.98	0.99	0.79	0.99	0.92	0.91	0.78	0.99	0.60	0.09	0.82	0.88	0.92	1.00		
17	0.92	0.90	0.93	0.95	0.77	0.95	0.88	0.90	0.74	0.95	0.57	0.08	0.78	0.84	0.96	0.98	1.00	
18	0.99	0.99	0.93	0.93	0.91	0.94	0.85	1.00	0.91	0.90	0.83	0.15	0.94	0.95	0.82	0.91	0.90	1.00

Annotation: The codes of chigger species are the same as in Table 5.

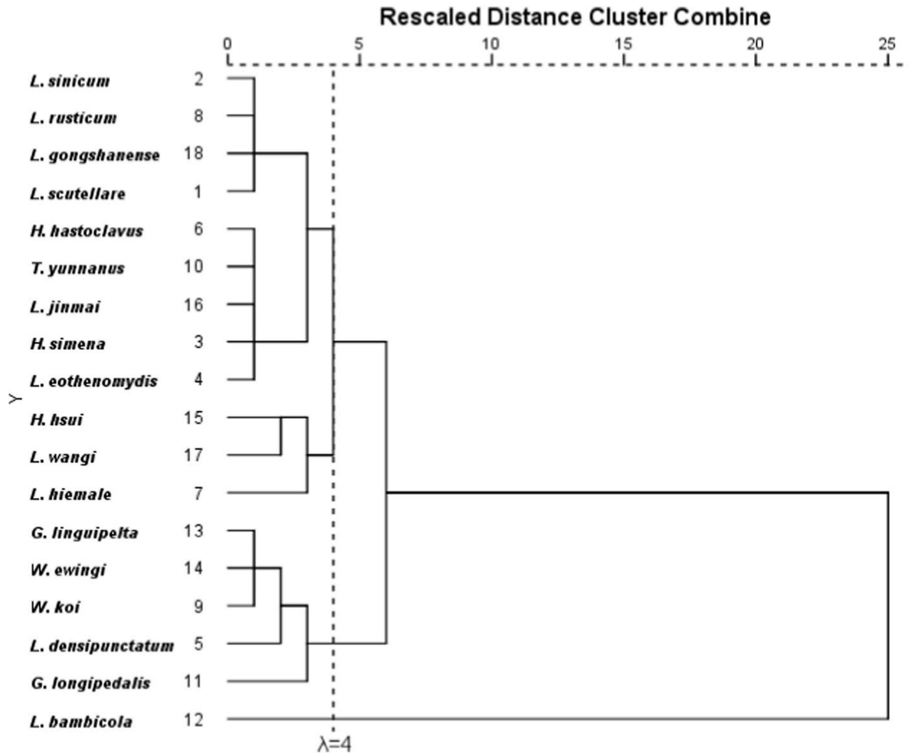

Fig. 3. The dendrogram of niche overlaps of the 18 main chigger species on large Chinese voles (*E. miletus*) along the combined environment series (multidimensional environment series) in the five provincial regions of Southwest China (2001–2019).

Table 8
The statistical parameters for fitting the theoretical curve of species abundance distribution of chigger mites on large Chinese voles (*E. miletus*) in five provincial regions of Southwest China (2001–2019).

Log intervals	Individual ranges in each log interval	Midpoint values of each individual range	Actual chigger mite species	Theoretical chigger mite species
0	0–1	1	29	25.40
1	2–4	3	32	31.61
2	5–13	9	34	34.00
3	14–40	27	26	31.61
4	41–121	81	31	25.40
5	122–364	243	10	17.64
6	365–1093	729	12	10.59
7	1094–3280	2187	6	5.50
8	3281–9841	6561	4	2.46
9	9842–29254	19683	1	0.96

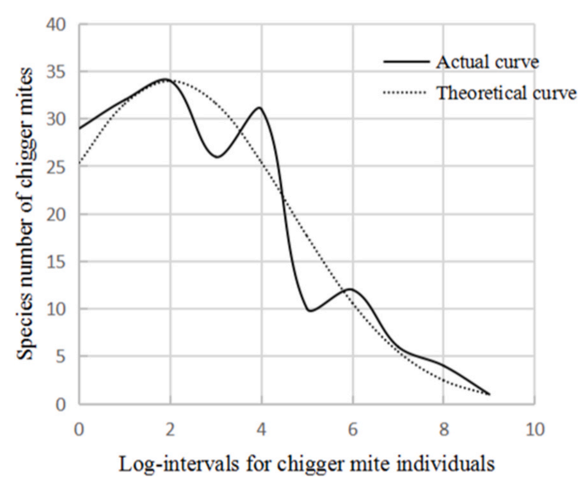

Fig. 4. The species abundance distribution of chigger mites on large Chinese voles (*E. miletus*) fitted by Preston's lognormal distribution model with the theoretical equation of $\widehat{S}(R) = 34e^{-[0.27(R-2)]^2}$.

higher infestation of chiggers on the male *E. miletus* than the females may be partially due to the relatively low resistance of the males. It is claimed that the males of rodents and other mammals usually have a lower resistance against parasites than the females because of the negative influence of androgen (male sex hormone) and the consumptive competition in mating activity (Folstad and Karter 1992; Xiang et al., 2021).

4.2. Dominant chigger species and their spatial distribution pattern and interspecific relationship on E. miletus

Leptotrombidium scutellare and *L. sinicum* were two dominant chigger species on *E. miletus* in Southwest China, which accounted for 30.23% (C_r = 30.23%) of the total 185 chigger species. It has been proved that there are six main vectors of scrub typhus in China, and these six vector chigger species are *L. deliense*, *L. scutellare*, *L. rubellum*, *L. sialkotense*, *L. wenense* and *L. insulare* (Li et al., 1997; Wu et al., 2013; Xiang and Guo 2021; Xiang et al., 2022). The occurrence of *L. scutellare* with the highest C_r on large Chinese voles (*E. miletus*) in Southwest China would increase the potential risk of chiggers' transmitting the pathogen of scrub typhus, *O. tsutsugamushi*, from voles to humans in the region. There is no evidence that *L. sinicum* can transmit *O. tsutsugamushi* effectively, and therefore we are not able to determine the epidemiological significance of *L. sinicum* in the present paper.

The spatial distribution pattern is an important issue in ecological practice with a lot of methods to measure it. The patchiness index (m^*/m), clump index (I) and Cassie index (C_A) used in the present study are the commonly used methods (Liu et al., 2019; Ding et al., 2021). The calculated values of m^*/m, I and C_A for two dominant chigger species

(*L. scutellare* and *L. sinicum*) were much higher than the border values of determining an aggregated pattern ($m^*/m > 1$, $I > 0$, $C_A > 0$) (Table 3), and therefore the two dominant chigger species were determined to be of aggregated distribution among different individuals of their host, *E. miletus*. The aggregated distribution pattern suggests that chiggers are not evenly distributed among different individuals of a certain host species (e.g., *E. miletus* in the present paper), and they may occur with clumps of mites on some host individuals and few (or no) mites on some other host individuals. The aggregated distribution pattern is often considered beneficial to the survival, fertilization, reproduction and defense of parasite populations (Shaw and Dobson 1995; Liu et al., 2019).

The interspecific relationship between any two different species or among many species is a complex issue in ecology (Fenton et al., 2010; Verbruggen et al., 2012). With the combination of Chi-square test (x^2), the present study used Pearson correlation coefficient (*PCC*), Dice coefficient (*DI*) and Ochiai coefficient (*OI*) to quantitatively measure the interspecific association between two dominant chigger species. The *ad* value was much higher than *bc* value for two dominant chigger species, *L. scutellare* and *L. sinicum* (Table 4), and this result qualitatively indicates that a positive association exists between two chigger species. The higher values of *PCC*, *DI* and *OI* (>0.5) quantitatively reveal a higher positive association between two dominant chigger species. The positive association suggests that two chigger species tend to co-exist on the body surface of the same host. This coexistence reflects the same host selection and mutual attraction between two species (Peng et al., 2016b; Zhang et al., 2022), which is probably an interspecific cooperation instead of interspecific competition (Verbruggen et al., 2012; Zhang et al., 2022). However, the related mechanism of the mutual attraction and interspecific cooperation remains to be further studied.

4.3. Niche characteristics of main chigger species in different environments

As two important ecological concepts and parameters, the niche width and niche overlap are often used to evaluate the extent to which a certain species uses a series of resources and the overlapped degree to which different species use the same series of resources (Futuyma and Moreno 1988; Yu et al., 2022; Zhang et al., 2022). When a group of different species of animal hosts are regarded as a certain series of host resources, the niche breadth can be used to compare the host specificity of different species of chiggers and other ectoparasites, and the niche overlap can be used to evaluate the overlapped extent of different species in the selection of different host species (Krasnov et al., 2005; Peng et al., 2017). The present study, however, involved only one species (*E. miletus*), and the niche breadth was used to compare the utilized extent of different chigger species along different environment gradients (environment series) instead of comparing their host specificity. Most chigger species have low host specificity. A certain chigger species can parasitize different animal hosts and a certain host species can harbor different chigger species as well (Li et al., 1997; Peng et al., 2016a; Xiang and Guo 2021). The distribution of chiggers is often influenced by a series of factors including animal hosts and environments, and the host and environment selection of chiggers are different from species to species (Chen et al., 2021b, 2022; Ding et al., 2021). On the same host species, the niche breadths of different chigger species can reflect their different ecological adaptability to different environments. The species with wide niche breadths usually have strong adaptability to their environments. On the contrary, the species with narrow niche breadths often have weak adaptability to their environments, and the weak adaptability and fragile competition can easily cause the extinction of the species (Yang et al., 2019; Chen et al., 2021a). The high niche breadths of *L. densipunctatum*, *W. koi*, *H. hsui*, *L. scutellare* and *W. ewingi* (Table 5, Fig. 2) suggest that these chigger species have a high adaptability to their environments with a wide distribution scope. The high adaptability of *L. scutellare* (a main vector species in China) to the environments would increase the potential risk of transmitting scrub typhus in different regions. In the clustering dendrogram, the 18 main chigger species were classified into 4 groups, and the different chigger species within the same group had a high degree of niche overlaps (Table 7, Fig. 3). The high niche overlaps suggest that these chigger species tend to choose the same or similar environments with similar environmental adaptability. The results further imply that some chigger species can coexist not only on the same species of host, but also in the same environment, and this may be an interspecific cooperation (Peng et al., 2016a; Zhang et al., 2022).

4.4. Species abundance distribution and total expected species of chiggers on E. miletus

In ecological studies, the species abundance distribution is to illustrate the relationship between the individuals and species in a certain community, and Preston's lognormal model is often used to fit the theoretical curve of the species abundance (McGill et al., 2007; Ding et al., 2021). In the present study, the species abundance distribution of chiggers on *E. miletus* was successfully fitted with Preston's lognormal model and the theoretical equation was $\widehat{S}(R) = 34e^{-[0.27(R-2)]^2}$ (Table 8, Fig. 4). The curve tendency of the species abundance distribution showed a gradual descending tendency from log interval 2 to log interval 8 and 9. Log interval 2 corresponded to the highest number of chigger species with 5–13 individuals, and log interval 8 and 9 corresponded to the few dominant chigger species with abundant individuals (Table 8, Fig. 4). The result is highly consistent with the previous study, which indicates that the majority of chigger species belong to rare or uncommon species with few individuals and few species are dominant ones with abundant individuals (McGill et al., 2007; Liu et al., 2019; Ding et al., 2021).

In community ecology, how to scientifically estimate the total number of expected species is often necessary and important, but it is very difficult to make an accurate estimation. There are a lot of methods to estimate the total expected species, and one of them is the formula $S_T = (S_0\sqrt{\pi})/\alpha$ based on Preston's model (Preston 1948; Ding et al., 2021). According to the calculated result, the expected total species of chiggers on *E. miletus* was estimated to be 223 species ($S_T = 223$), 38 more than actually collected species (185 species). The estimated result implies that 38 species have been probably missed in the actual field investigation. In fact, some rare species are unavoidable to be missed in field investigations because they are too few to be collected in sampled investigations (Baltanás 1992; Ding et al., 2021).

5. Conclusions

The large Chinese vole (*E. miletus*) has a great potential to harbor lots of chiggers with high species diversity and infestation in the five provincial regions of Southwest China. The male voles have higher infestation than the females with sex-bias. *Leptotrombidium scutellare* and *L. sinicum* are two dominant chigger species on *E. miletus* and they are of aggregated distribution among different individuals of *E. miletus*. A positive association exists between two dominant chigger species. *Leptotrombidium densipunctatum*, *W. koi*, *H. hsui*, *L. scutellare* and *W. ewingi* have a strong adaptability to their environments with high niche breadths and some chigger species tend to coexist in similar environments with obvious niche overlaps. Based on the theoretical curve fitting of species abundance, the expected total number of chigger species can be roughly estimated.

Declaration of competing interest

All the authors declare that there is no conflict of interest.

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Appendix. The captured large Chinese voles (*Eothenomys miletus*) from each of 91 investigation sites in the five provincial regions of Southwest China (2001–2019)

Site abbr.	Names of investigation sites	Captured voles	Site abbr.	Names of investigation sites	Captured voles	Site abbr.	Names of investigation sites	Captured voles
AY▲	Anyue	0	JY▲	Jiangyang (Leshan)	0	SM	Simao	0
BC*	Binchuan	34	KR▲	Karuo (Changdu)	0	SN▲	Sinan	0
BY▲	Bayi (Linzhi)	0	LC*	Longchuan	131	SZ▲	Shizhu	0
CS▲	Changshou	0	LH*	Lianghe	129	SZh▲	Shizhong (Leshan)	0
CY*	Cangyuan (Lincang)	2	LHo▲	Luhuo	0	TN▲	Tongnan	0
DC	Daocheng	0	LL*	Luliang	2	TZ▲	Tongzhi	0
DJ▲	Dianjiang	0	LLi*	Longli	12	WS	Wenshan	0
DL*	Dali	1081	LP*	Lanping	13	WuS▲	Wusheng	0
DQ*	Deqin	52	LS▲	Lushui	0	WX*	Weixi	430
DY▲	Daying	0	LX▲	Luxian	0	WY▲	Weiyuan	0
DYu*	Duyun	68	LZ▲	Lezhi	0	WZ▲	Wanzhou	0
FC▲	Fucheng (Mianyang)	0	MEK▲	Maerkang	0	XC	Xiangcheng	0
FG▲	Fugong	0	MG	Maguan	0	XGLL	Xianggelila	0
FL▲	Fuling	0	MH	Menghai	0	XH▲	Xuanhan	0
FY*	Fuyuan	12	MK▲	Mangkang	0	XX*	Xixiu (Anshun)	32
GD▲*	Guiding	23	ML▲	Mengla	0	XZ▲	Xuzhou (Yibin)	0
GL▲*	Guanling	1	MLi*	Muli	85	YaJ▲	Yajiang	0
GM*	Gengma	3	MN▲*	Mianning	12	YD*	Yongde	106
GS*	Gongshan	18	MY*	Miyi	19	YJ	Yuanjiang	0
GZ▲	Ganzi	0	MZ	Mengzi	0	YL*	Yulong Lijiang	19
HK	Hekou	0	NE	Ninger	0	YoY▲*	Youyang	2
HS▲*	Huishui	4	PA▲*	Puan	103	YuY▲	Yunyang	0
HX*	Huaxi (Guiyang)	48	PC▲	Pingchang	0	YY*	Yanyuan	30
HY▲	Hongya	0	PS▲	Pingshan	0	ZA▲	Zhengan	0
JC*	Jianchuan	83	QB	Qiubei	0	ZF▲	Zhenfeng	0
JH	Jinghong	0	QJ	Qiaojia	0	ZJ*	Zhijin	50
JJ▲	Jiangjin	0	QW▲	Qianwei	0	ZS▲*	Zhongshan (Liupanshui)	17
JK▲	Jiangkou	0	RJ▲	Rongjiang	0	ZX▲	Zhongxian	0
JP▲*	Jinping	4	RL*	Ruili	36	ZZ▲	Zizhong	0
JS▲	Jinsha	0	RS▲	Renshou	0			
JT▲	Jintang	0	SJ	Suijiang	0			

(Annotation: Site abbr. = the name abbreviations of the investigation sites. The investigation sites marked "▲" were newly increased sites after 2013 and those marked "*" were the sites where large Chinese voles, *Eothenomys miletus*, were captured).

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